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Poul Brændstrup

25 years with Acta

Professor Poul Brændstrup has taken part in the editing of *Acta Ophthalmologica* for 25 years first as editorial secretary and later as chief editor

This very comprehensive and time consuming work gave Brændstrup a feeling of being so to speak wedded to *Acta*

Brændstrup's criticism of the manuscripts has always been positive and constructive There is hardly any author who does not feel under an obligation to him His kindness his readiness to help and his profound knowledge of all aspects of ophthalmology are legendary

Brændstrup wishes to retire from his post as chief editor He will be greatly missed but fortunately he has decided to continue as Danish deputy editor – he still has a deep affection for *Acta*

This journal owes a great debt of gratitude to Poul Brændstrup who devoted his leisure time to this work

Brændstrup is irreplaceable – it has therefore been decided to alter the structure of *Acta* In future the work will be carried out by ten local editors and two international editors while the new chief editor will have mainly co ordinating and publishing tasks

Mogens S Norn

University Eye Department (Head Professor Thore Lie Thomassen) Rikshospitalet Oslo Norway
and the Institute of Pathology Electron Microscopic Laboratory (Head T Hovig M D) Rikshospitalet Oslo Norway

PSEUDO EXFOLIATION MATERIAL ON THE ANTERIOR LENS SURFACE DEMONSTRATION AND EXAMINATION OF AN INTERFIBRILLAR GROUND SUBSTANCE

BY

MARTIN DAVANGER and OLAV OYVIND PEDERSEN

Peroxidase did not penetrate into the capsule of cataractous lenses with or without pseudo exfoliation (PE) Neither did this tracer penetrate into the PE material itself indicating that the PE fibrils are embedded in a ground substance which is impenetrable to peroxidase Staining with alcian blue and ruthenium red showed that this ground substance is structurally heterogeneous Acid mucopolysaccharides and/or glycoproteins are probably present The fibrils are coated by a material with affinity to ruthenium red This applies also to the fibrils of the amorphous layer Their diameter is only about 1/3 of the fibrils of the PE material on the lens surface Rounded membrane covered bodies are present partly in groups in the PE material Morphologically they resemble the mucopolysaccharide containing lysosomal vacuoles found in systemic mucopolysaccharidoses Observations supporting the conception of similarities between PE material and amyloid *are pointed out The pathogenesis of the PE material* is discussed

Key words lens – lens capsule – pseudo exfoliation – permeability – peroxidase – alcian blue – ruthenium red – mucopolysaccharide – amyloidosis

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The pseudo exfoliation (PE) material on the anterior lens surface is described as consisting of straight irregularly outlined fibrils distributed at random It has been proposed that the material originates from the lens epithelial cells which in this condition produce a fibrillar substance This substance is supposed to pass the fibrillar layer of the capsule as granules get their fibrillar structure again as they reach the surface and are poured out on the surface of the lens capsule where they form the characteristic bush shaped excrescenses (Bertelsen et al 1964)

This theory presupposes that the lens capsule in this condition is permeable to formed elements or high molecular substances (Ashton et al 1965 Benedikt 1973)

The present work was initiated on this basis with the purpose of examining the permeability of the lens capsule to a protein tracer horseradish peroxidase

As will be demonstrated and discussed it became apparent that the tracer did not penetrate into the PE material itself From this it was concluded that the fibrils are embedded in a ground substance which is impermeable to peroxidase molecules The aim of our work was therefore extended to include a further examination of this ground substance

Material and Methods

Three cataractous lenses with PE and two without all obtained by cataract cryo extraction were used to examine the penetration of peroxidase The lenses were immediately suspended for 2 hrs in room temperature in a 0 25 % solution of commercial horseradish peroxidase (Sigma type II mol wt 40 000) in Ringer s solution After rinsing in isotonic phosphate buffer pH 7 4 the lenses were incubated for 2 hrs in room temperature in tris HCl buffered diaminobenzidine H O solution pH 7 6 (Karnovsky 1967) After new rinsing in buffer the lenses were fixed in 1 % OsO_4 in Millonig s phosphate buffer pH 7 4 and dehydrated in graded ethanol solutions

At this stage the lenses were carefully dissected under a dissecting microscope Specimens were prepared from the following zones 1 The central disc 2 The intermediate zone 3 The peripheral band 4 The equatorial region 5 The posterior surface

That part of the lens surface which had been frozen and deformed during the cryo extraction was located by inspection and discarded as preliminary examinations demonstrated marked artifacts at this location

The specimens were suspended in propylene oxide thereafter embedded in

Epon 812 and sectioned with LKB ultramicrotome Sections 1 μm thick un stained or stained with toluidine blue were examined with phase contrast light microscopy Some ultrathin sections were contrasted with alkaline lead or an aqueous solution of uranyl acetate or both Electron micrographs were taken with a Siemens Elmiskop 1A

Three other lenses with PE and two without were stained with alcian blue as described by Behnke & Zelander (1970) The lenses were suspended for 4 hrs in a solution of 4 % glutaraldehyde and 1 % alcian blue in 0 1 M cacodylate buffer pH 6 3 After rinsing in cacodylate buffer the lenses were postfixed for 2 hrs in 2 % OsO_4 in cacodylate buffer

Four other lenses with PE and two without were stained with ruthenium red according to methods described by Luft (1971) The lenses were suspended *en bloc* for 4 to 17 hrs in 2 % OsO_4 and 2 % ruthenium red in 0 1 M cacodylate buffer pH 7 3

These lenses were dehydrated dissected embedded sectioned and examined as described above

Results

Location of peroxidase No penetration of peroxidase into the lens capsule was found by light and electron microscopy either in the lenses with PE or in those without This applies to all zones examined (except for that part of the lens surface which had been frozen and deformed during the lens cryo extraction) Special emphasis was laid upon examining the lens capsule at the peripheral band and in the pre equatorial region The two layers which may be found here with their characteristic pattern of granules and fibrils (Bertelsen et al 1964 Ashton et al 1965 Dark et al 1969) were identified both by light and electron microscopy Any penetration of peroxidase into the capsule was firmly ruled out

At the outset it was thought that peroxidase would be kept in the spaces be tween the fibrils of the PF material which would become heavily stained in these specimens However an unexpected picture emerged *Peroxidase did not penetrate into the PE material but was attached to its surface where a dense coating appeared* This was the case both at the excrescences of the peripheral band and at the central disc and it was obvious both by light and electron micro scopy (Figs 1 and 2) The central disc did not stain with toluidine blue in these epon sections and its presence would have been overlooked by light microscopy without its peroxidase coating

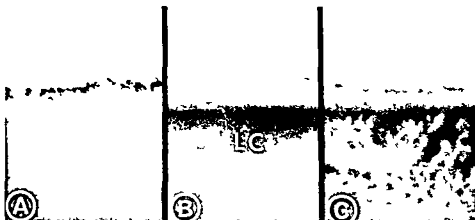


Fig 1

The central disc A Peroxidase coating toluidine blue B Stained with alcian blue C Stained with ruthenium red LC Lens capsule Light microscopy × 1 830

Fig 2

Granules of peroxidase reaction product (arrow) on the surface of the central disc (CD) LC Lens capsule Electron microscopy contrasted with lead and uranyl × 9 300

Obviously an interfibrillar substance prevented the penetration of peroxidase to the spaces between the fibrils

Staining of interfibrillar substance The obvious next step was to apply staining procedures aimed at a positive demonstration and further characterization of the interfibrillar matrix

In preliminary experiments fresh lenses with PF were suspended *en bloc* in Sudan black and Oil Red O solutions for varying time up to 4 hrs After rinsing in isotonic buffer the whole lenses were examined under a dissecting microscope No staining of the PE material was observed It is concluded that lipids are probably not a substantial part of the PE material

Mucopolysaccharides constitute a major part of the ground substance of fibrillar tissues and it was found natural to apply dyes with affinity to these substances Lenses were stained *en bloc* with alcian blue and ruthenium red as described above The staining methods are applicable both to light and electron microscopy

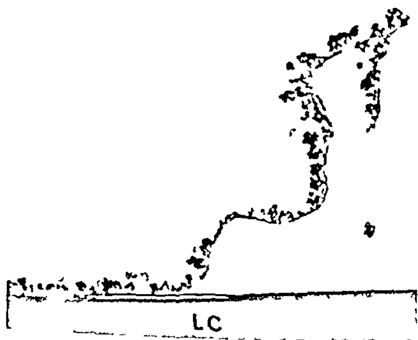


Fig 3

Pseudo exfoliation, peripheral band Excrescenses resting on a basal lamina which has been partly lifted up from the lens capsule (LC) Light microscopy alcian blue × 7

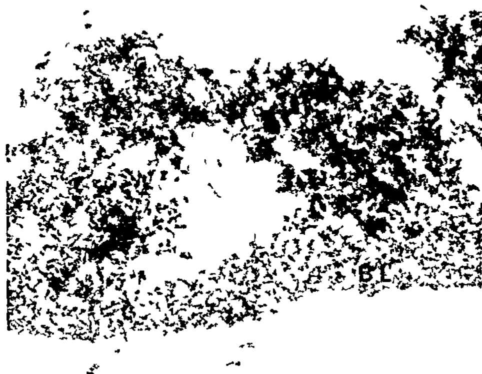


Fig 4

Pseudo exfoliation peripheral band the same area as in Fig 3 The excrescenses are resting on a basal lamina (BL) which has been lifted up from the lens capsule Most of the ground substance has been stained Electron microscopy alcian blue uncontrasted × 16 750

Light microscopy showed that both alcian blue and ruthenium red stained the PE material both at the peripheral band (Fig 3) and the central disc (Fig 1) The PE material is not stained by toluidine blue in ordinary OsO_4 fixed and epon embedded sections but obtains affinity to toluidine blue after the application of alcian blue and ruthenium red as described

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Fig 5

Pseudo exfoliation material Rounded membrane covered body with fibrillo granular content in upper right corner Coating of the fibrils Woolen lines LC Lens capsule Electron microscopy ruthenium red uncontrasted × 67 000


LC

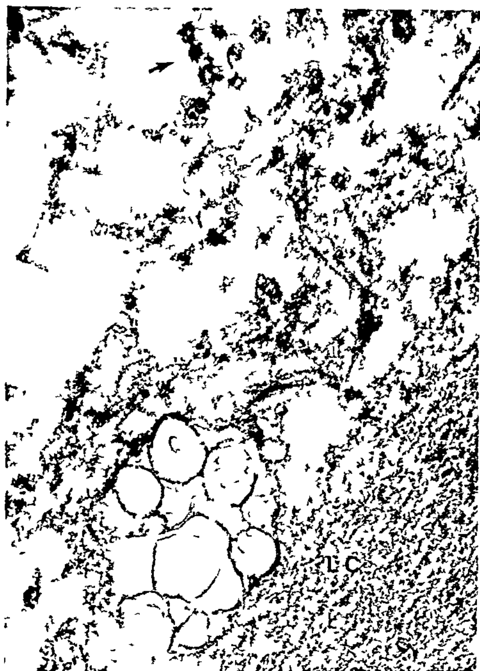


Fig 6

Pseudo exfoliation material A group of rounded bodies resting on the lens capsule (LC) A membrane surrounds the whole group Trilaminar membranes are seen Coating of the PE fibrils (arrow) Electron microscopy ruthenium red uncontrasted × 70 000

Fig 3 demonstrates that the bush shaped excrescenses of the peripheral band may rest upon a continuous basal lamina This lamina may occasionally be de tached from the lens surface as a continuous membrane while the excrescences remain intact on the surface of the membrane

Electron microscopy revealed further details A major part of the material was stained by alcian blue (Fig 4) However the staining was uneven and some areas remained unstained In some parts of the material no regular pattern was traced Other parts mainly in the basal lamina seemed to be built up by small unstained rounded areas surrounded by more or less distinct lines In uncon trasted sections the fibrils of the PE material were seen only faintly

Additional information on the composition of the interfibrillar material was obtained from the specimens stained with ruthenium red *The fibrils were seen to be coated by a material with affinity to ruthenium red* and they appeared as tubes in the uncontrasted sections (Figs 5 6 and 7) Cross sectioned fibrils were seen as small circles surrounding lighter areas The diameter of the fibrils in cluding their coating was found to be about 400–500 Å

A number of *rounded bodies were found within the PE material* (Figs 5 6 and 7) They are surrounded by a densely stained line and contain a fibrillo granular lightly stained material Inclusions of irregular or annular form may be present The rounded bodies have a size varying between 0 2 and 0 5 μm Some of the bodies are collected in groups (Figs 6 and 7) which when considered in three dimensions seem to contain more than 30–40 bodies In the group seen in Fig 6 a trilaminar membrane is observed at certain sites and the group of bodies is enclosed in a membrane surrounding the whole group

A meshwork of woolen lines appeared in some parts of the PE material mainly in its deeper parts (Fig 5) The woolen lines delineate unstained areas which have a size comparable to the rounded bodies The fibrils tend to be arranged along the woolen lines

Also the fibrils in the amorphous layer of the lens capsule were coated by a material with affinity to ruthenium red (Fig 8) The coating was seen on both longitudinally and cross sectioned fibrils the latter appearing as small circles in uncontrasted sections The coating facilitated the measurement of the dia meter of these fibrils which was found to be approximately 150 Å the coating included This means that the fibrils of the amorphous layer have a diameter of only about 1/3 of the fibrils in the PE excrescenses on the lens surface

Most of the fibrils were collected in bundles as described by Bertelsen et al (1964) Ashton et al (1965) and Dark et al (1969) The bundles of fibrils were surrounded by an irregular unstained area, while the main part of the amorph ous layer had a fine granular staining



Fig 1

Pseudo exfoliation material Rounded bodies single and in a group Coating of the fibrils Electron microscopy ruthenium red uncontrasted × 37 500



Fig 8

A bundle of fibrils in the amorphous layer of the lens capsule Cross sectioned The bundle is surrounded by an electronlucent area Coating of the fibrils (arrow) Electron microscopy ruthenium red uncontrasted × 90 000

Discussion

The lens capsule is impenetrable to our protein tracer molecule even in the regions of the lens capsule where the amorphous deep layer is present This observation seems to weaken the conception that the PE material on the lens surface is produced by the lens epithelium and penetrates the lens capsule

The PE material on the lens surface has been described as consisting of fibrils (Blackstad et al 1960 Bertelsen et al 1964 Ashton et al 1965) and the presence and nature of an interfibrillar ground substance has not been discussed before

The results of our peroxidase penetration experiments do not leave any doubt about the presence of *an interfibrillar matrix as a major part of the PE material*

The tracer used in these experiments penetrates readily in the ground sub-

stance of connective tissues even through such dense tissue as the corneal stroma (Tonjum 1974) The lack of penetration into the PE material is therefore remarkable and may throw some light on the physico chemical properties of its ground substance Perhaps it may be an important factor in the resistance of the PE material to enzymatic digestion (Arnesen et al 1963)

Further information on the composition of the interfibrillar ground substance is obtained from the specimens stained with alcian blue and ruthenium red The matrix is structurally heterogeneous It seems to contain different chemical substances partly arranged in definite structures and formed elements The heterogeneous composition of the PE material may explain why histochemical investigations by light microscopy have been inconclusive with regard to the chemical nature of the PE material

Alcian blue and ruthenium red when applied as described is considered to stain acid mucopolysaccharides (Luft 1971 Behnke & Zelander 1970) Our results indicate that acid mucopolysaccharides probably are present in the ground substance of the PE material and may constitute a major part of it The coating of the fibrils of the PE material in the ruthenium red preparations may indicate that a glycoprotein is structurally orderly arranged along the fibrils

Several researchers have had difficulties in demonstrating the central disc by histological methods (Sunde 1956 Bertelsen et al 1964) and no convincing pictures have been published before the work of Benedikt et al (1973) These authors demonstrated the central disc as seen by scanning and transmission electron microscopy In our sections prepared with peroxidase and stained with toluidin blue the central disc would have been overlooked by light microscopy if it had not been delineated by the peroxidase line However the material of the central disc is stained and easily seen in the alcian blue and ruthenium red preparations (Fig 1)

The rounded bodies contained in the PE material (Figs 5 6 and 7) are thought to have an intracellular origin They have a striking similarity even in some of their details to the membrane limited intracytoplasmic vacuoles seen also in the epithelium of the ciliary body in mucopolysaccharidoses and mucolipidoses (Emery et al 1971 Kenyon & Sensenbrenner 1971 Kenyon 1974) Such vacuoles are considered to be altered lysosomes that have become engorged with acid mucopolysaccharide and glycolipid storage substances These substances are thought to accumulate because of a deficiency of hydrolytic enzymes This deficiency results inadequate degradation of substances which then progressively accumulate within lysosomal storage vacuoles

The morphological similarity between the rounded bodies in the PE material and the storage vacuoles in mucopolysaccharidoses may perhaps indicate a corresponding biochemical relationship If this is so the presence of these bodies

in the PE material may be considered as further indication of the importance of acid mucopolysaccharide in the pathogenesis of the PE syndrome

Ringvold & Husby (1973) have given histochemical immunological and ultra structural evidence indicating that the PE material should be classified among the amyloid like substances

Some of the results of the present work may support this conception

1 Amyloid material consists of pathological deposits of tissue in which pro tein fibrils are embedded in an interfibrillar matrix which contains mucopoly saccharides (Muir & Cohen 1968) In the present work evidence has been given that this description holds also for the PE deposits of the lens surface

2 The amyloid fibrils are coated by a substance with affinity to ruthenium red (Gueft 1968) As shown above this is the case also for the PE fibrils

3 *Lanthanum hydroxide* does *not* penetrate into the masses of amyloid even in areas between fibrils where there would appear to be a potential space (Soren son & Bari 1968) This observation was taken as evidence that the amyloid deposits consist of additional material which could not be recognized by electron microscopy The material occupies the space between the fibrils and limits the penetration of the tracer molecule

A similar observation on the PE material is described in the present work.

4 Ringvold & Husby (1973) pointed out that a difference between amyloid and PE fibrils is their different diameter 100 Å vs 200–375 Å respectively However the present work shows that the fibrils of the amorphous layer found *in the lens capsule* as part of the PE syndrome have a diameter similar to that of the amyloid fibrils

5 In amyloid deposits two fibrils are frequently seen to be associated side by side (Sorenson & Finke 1968 Sorenson & Bari 1968) In some of our sections of PE material the same phenomenon has been observed

6 Sorenson & Bari (1968) have observed rounded bodies from 500 Å to 2 000 Å in diameter in the amyloid tissue Their bodies have some similarity to the bodies described above in the PE material

7 The PE material is highly resistant to proteolytic enzymes (Arnesen et al 1963) This is also the case for amyloid deposits (Sorenson & Binington 1964)

On the basis of the conception of a relationship between the PE and amyloid material it seems relevant to see if information obtained from the literature on amyloidosis may help to elucidate the nature and the pathogenesis of the PE syndrome

Schwartz (1968) finds cerebral and cardiovascular amyloid deposits to be a frequent finding in the aged The cerebral deposits are found on the cortical surface on the surface of the cerebral ventricles and in the wall of cerebral vessels The heart the aorta and the Langerhan s islets of the pancreas are frequently involved

If the relationship between amyloid and PE is confirmed by further research it may be that intraocular surfaces and the wall of iris vessels should be added to this list of typical locations for amyloid deposits in the aged

The pathogenesis of amyloidosis is not well known in spite of extensive research It is thought that the amyloid material is produced by cells *in situ* at the site where it is found (Teilum 1968 Christensen 1968 Sorenson & Bari 1968 Cohen 1968) Reticulo endothelial cells and also endothelial and epithelial cells are thought to be able to produce the amyloid fibrils (Sorenson & Bari 1968 Gueft & Ghidoni 1968) The mucopolysaccharides of the ground substance in connective tissue may be of importance in the formation of amyloid Chemical and physical alterations of the ground substance may precede the amyloid formation and be the cause of precipitation of amyloid protein fibrils from soluble precursors (Catchpole 1968 Muir 1968 Christensen 1968)

It is tempting to speculate that a similar mechanism is involved in the formation of the PE material Mucopolysaccharides seem to be an important part of its ground substance Accumulation of mucopolysaccharides (caused by an enzymatic defect ?) may cause the precipitation of the protein fibrils of the material This process may take place simultaneously at different locations of the anterior part of the eye The PE material on the anterior hyaloid on the zonules and on the anterior lens surface may be brought to its position through the aqueous

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THE SUSPENSORY APPARATUS OF THE LENS THE SURFACE OF THE CILIARY BODY

A scanning electron microscopic study

BY

MARTIN DAVANGER

Normal anterior segments from five human eyes were prepared for scanning electron microscopy by fixation in glutaraldehyde and OsO_4 careful dissection drying and coating The lens equatorial region the zonules and the ciliary body were examined from behind and front The zonules were found to insert at circular lines immediately in front of and behind the equator of the lens and some directly on to the equator In the ciliary body they inserted along the ridges of the processes Their insertions by ramification are studied in detail No zonules were found to insert into or pass through the ciliary valleys nor did they insert on pars plana or ora serrata Short zonule like strands connected the posterior end of the processes with pars plana and a mat consisting of confluent zonule like fibers running in a meridional direction, covered the surface of pars plana The zonules inserting behind the lens equator seemed to adhere to the anterior face of the vitreous but they did not merge into it

Key words zonules of Zinn – lens – ciliary body – vitreous – accommodation – scanning electron microscopy

Details of the gross anatomy of the zonules and their relation to neighboring structures are difficult to establish This part of the eye has a very complicated macroscopic architecture The tissues are delicate and of varying texture It is difficult to reconstruct the three dimensional anatomy of this region from microscopic sections The value of an examination with a dissecting microscope is limited because of the relative invisibility of the vitreous and the zonules and

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also because of the low magnification and poor depth resolution inherent in this method

The insertion and course of the zonules and also their relations to neighboring structures is therefore still in dispute Inconsistent information is obtained from the literature

Most authors (McCulloch 1954 Vail 1957 Rohen & Rentsch 1969 Hogan et al 1971) assert that the majority of zonular fibers arise from the region of ora serrata or from pars plana ciliaris (Raviola 1971) It is stated that the zonules course forward from ora serrata hugging the concavity of pars plana being pushed into place by the vitreous When reaching the posterior margin of the ciliary processes they separate into groups which run forward in the valleys be tween the processes and supporting fibers merging with the main system of zonules arise in the valleys It is firmly asserted that the zonules do not insert on the crest of the ciliary processes (for ref see Vail 1957)

On the other hand Kaczurowski (1964) found that the posterior zonules emerge from the ciliary processes also from their apices while the anterior zonules come from the bottom of the ciliary valleys and at times from pars plana

It was thought that scanning electron microscopy might be a valuable method for the examination of this region The scanning electron micrographs have the advantage of giving information on the three dimensional architecture of con volute surfaces The depth resolution is increased about 200 times as compared with incident light micrographs of the same magnification The magnification may be varied within a wide range without changing the position of the speci men whereby pictures taken with high magnification are easily localized on the specimen

The purpose of this work is to study the detailed gross anatomy of the zonules and the surfaces of their neighboring structures by the help of the scanning electron microscope

Material and Methods

Five eyes from adult humans were examined Three of these were enucleated because of a choroidal melanoma affecting the posterior segment only while two were donor eyes taken from the same person The former were processed immediately after enucleation while the latter were enucleated some hours *post mortem* and kept in a moist chamber at 4°C a few hours before processing The eyes were carefully cut along equator The anterior halves were prefixed for 2 hours in 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 After rinsing in isotonic buffer the specimens were postfixed for 2 hours in 1 % OsO_4 in Millonig s phosphate buffer pH 7 4 and dehydrated in graded ethanol solutions

At this stage the vitreous of three eyes was carefully removed from behind under a dissecting microscope while the eyes were submerged in ethanol The vitreous was firmly adherent to the region of ora serrata but after being torn at this site the anterior face of the vitreous was only loosely adherent to pars plana the posterior zonules and the lens and could be lifted off in most cases in a continuous membrane (as was later confirmed by a scanning electron microscopy of the anterior face of the vitreous to be published) The careful removal of the vitreous did not cause any visible damage to the zonules

The sclera and cornea were removed in one piece by careful dissection with a spatula inserted between the sclera and the ciliary body by a procedure similar to that used in cyclodialysis In two eyes in which the vitreous remained the iris was removed by tearing along the iris root These two eyes were viewed from the front in the scanning electron microscope One of the two donor eyes was seen from the front while its fellow eye was examined from behind

The two first processed eyes were dryed in air at this stage It was found however that because of an uneven shrinkage of the different tissues the ciliary body and the zonules became irregularly deformed during the drying process Therefore only parts of the specimens could be examined and a general view could not be obtained To avoid this deformation the following procedure was applied to the remaining three eyes The specimens were suspended in propylene oxide thereafter in Epon 812 and kept in room temperature for 2 days They were then washed in propylene oxide under a dissecting microscope with the help of a pipette until the Epon solution seemed to be completely washed away from the surfaces It is thought that some partly polymerized Epon forming a denser skeleton remained within the tissue The specimens thus treated were less deformed during the following air drying process Some details of the surface as seen in high magnification may be influenced by this procedure but it is felt that serious artifacts are not introduced in the gross anatomy of the specimens

The specimens were air dryed in room temperature for 3 days The first part of the drying process was followed by the help of a dissecting microscope The specimens were mounted on specimen holders and coated in a vacuum with a thin layer of carbon and gold palladium Scanning electron microscopy was performed with a Jeol JSM 50 A scanning electron microscope

Results

Fig 1 gives a general view of part of a specimen seen from behind The zonules bridge the space between the lens equator and the ciliary processes They tend to be collected in groups which are directed towards the processes The zonules

also because of the low magnification and poor depth resolution inherent in this method

The insertion and course of the zonules and also their relations to neighboring structures is therefore still in dispute Inconsistent information is obtained from the literature

Most authors (McCulloch 1954 Vail 1957 Rohen & Rentsch 1969 Hogan et al 1971) assert that the majority of zonular fibers arise from the region of ora serrata or from pars plana ciliaris (Raviola 1971) It is stated that the zonules course forward from ora serrata hugging the concavity of pars plana being pushed into place by the vitreous When reaching the posterior margin of the ciliary processes they separate into groups which run forward in the valleys between the processes and supporting fibers merging with the main system of zonules arise in the valleys It is firmly asserted that the zonules do not insert on the crest of the ciliary processes (for ref see Vail 1957)

On the other hand Kaczurowski (1964) found that the posterior zonules emerge from the ciliary processes also from their apices while the anterior zonules come from the bottom of the ciliary valleys and at times from pars plana

It was thought that scanning electron microscopy might be a valuable method for the examination of this region The scanning electron micrographs have the advantage of giving information on the three dimensional architecture of convolute surfaces The depth resolution is increased about 200 times as compared with incident light micrographs of the same magnification The magnification may be varied within a wide range without changing the position of the specimen whereby pictures taken with high magnification are easily localized on the specimen

The purpose of this work is to study the detailed gross anatomy of the zonules and the surfaces of their neighboring structures by the help of the scanning electron microscope

Material and Methods

Five eyes from adult humans were examined Three of these were enucleated because of a choroidal melanoma affecting the posterior segment only while two were donor eyes taken from the same person The former were processed immediately after enucleation while the latter were enucleated some hours *post mortem* and kept in a moist chamber at 4°C a few hours before processing The eyes were carefully cut along equator The anterior halves were prefixed for 2 hours in 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 After rinsing in isotonic buffer the specimens were postfixed for 2 hours in 1 % OsO_4 in Millonig s phosphate buffer pH 7 4 and dehydrated in graded ethanol solutions



Fig 2

Area indicated in Fig 1 (left) in higher magnification The insertion of groups of zonules along the ridge of two ciliary processes The zonules ramify along the epithelial surface In the valley (V) between the main processes thin strands are extended between small irregular processes × 190 Bar = 0 1 mm

thin out and disappear in the posterior part of the processes (Fig 1) Some strands of the fan may bridge over to neighboring processes or to small irregular processes situated in the valleys between two main processes (Fig 3) These small processes are often connected by thin strands which do not originate from the main or true zonules (Fig 2)

In the region of the ciliary processes and the valleys the surface is very convolute

Zonule like short fibers bridge between the posterior end of the processes and pars plana (Figs 1 and 4) These fibers are not continuous with the zonules which attach to the lens Anteriorly they split up in a fan and attach to the posterior part of the processes in much the same way as was described for the zonules coming from the lens Posteriorly these fibers merge into the surface of pars plana This surface is covered by a continuous mat of confluent long fibers running in a meridional direction (Fig 5) A few strands attached to the surface

insert along the ridge and slope of the processes They do not pass through or insert in the valleys as most earlier authors have described (Vail 1957 Rohen & Rentsch 1969 Hogan et al 1971) At the site of insertion the zonules split up in a number of fibrils (Figs 1 2 and 3) which can be followed as distinct entities along the surface of a considerable part of the processes The fibrils gradually

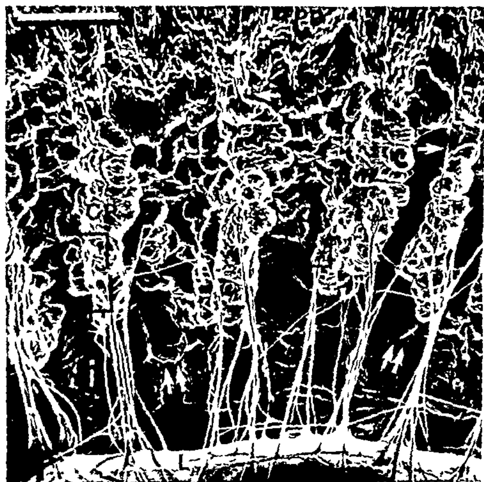


Fig 1

General view of a part of the posterior equatorial region of the lens (L) the zonules the iris (i) the ciliary processes (CP) and pars plana ciliaris (PP) The zonules are partly collected in irregular bundles directed towards the processes where the zonules insert mainly along their ridges Short zonule like strands (arrows) connect the posterior end of the processes with the anterior part of pars plana Zonula like strands insert on the posterior iris surface (double arrows) Areas indicated by right angles are seen in higher magnification in Figs 2 and 3 × 49 Bar = 0 5 mm



Fig 4

The posterior parts of the ciliary processes and the anterior part of pars plana ciliaris (PP) The posterior end of the processes are connected with short zonule like strands to the anterior part of pars plana × 120 Bar = 0 1 mm

In the eyes examined from the front the anterior zonules are seen to attach to the anterior equatorial region of the lens along a circular line in much the same way as the posterior zonules (Fig 9) The anterior zonules are directed towards the anterior end of the ciliary processes where they attach to the anterior part of their ridges In Fig 9 the anterior face of the vitreous can be seen in the background The zonules from the posterior lens surface are seen to lie against the vitreous face to which they seem to adhere but they do not seem to merge into it

In addition to the zonules which insert along a circular line on the anterior and posterior equatorial region of the lens some zonules insert directly on the

of this mat cross the main fiber direction They may end rather abruptly ramifying into a short fan (Fig 5 arrow and inset)

Turning towards the attachment of the zonules to the lens (Fig 6) the zonules are seen to split up into a few thinner units a short distance above the lens surface Here the zonules ramify further into thinner and thinner fibrils (Fig 7) The fans lie flat on the lens surface and broaden until they merge with neighboring fans and form a continuous mat of thin fibrils directed towards the lens pole (Figs 8 and 9) As seen from the surface the mat of fibrils terminates by *the fibrils gradually becoming thinner (Fig 10)*



Fig 8

Area indicated in *Fig 1 (center) in higher magnification* Detail of the insertion of zonules on the convolute surface of ciliary processes × 465 Bar = 50 μm

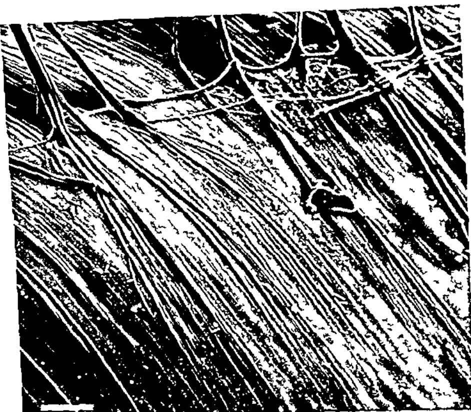


Fig 7

Area indicated in Fig 6 in higher magnification Ramification of the zonules at their insertion on the posterior lens surface Meridional striation of the lens equatorial region × 130 Bar = 0 1 mm

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Fig 5

Pars plana ciliaris The surface is covered by a continuous mat consisting of confluent zonule like fibers running in a meridional direction. A few superficial fibers cross the main fiber direction Inset demonstrates the ramification of the end of one of the cross ing fibers (at the arrow) × 130 inset × 690 Bar = 0 1 mm in inset Bar = 20 μm.

Fig 6

The insertion of the zonules on the equatorial region of the lens Oblique view from behind This area is seen directly from behind in Fig 1 (lower part central and right) Most zonules insert along a line parallel to the lens equator some insert directly on the equator Area indicated by right angles is seen in higher magnification in Fig 7 × 77 Bar = 0 2 mm

Fig 5



Fig 6

the edge of the sheet seen in Fig 11 is examined in high magnification it can not be seen that fibrils enter into the lens capsule

The diameter of the zonules is found to vary up to 40–50 μm in these specimens Their cross section seems to be circular and their surface is usually smooth and even also in high magnification However the zonules may have longitudinal stripes or grooves consistent with their being composed of thinner subunits These subunits split apart in the ramification near the insertion of the zonules and they can also split from one zonule to merge with a neighboring zonule



Fig 9

The anterior equatorial region of the lens the zonules the anterior face of the vitreous (V) and the anterior end of the ciliary processes The posterior zonules lie upon and seem to adhere to the anterior surface of the vitreous (arrow) The anterior zonules insert near the anterior end of the ciliary processes ×67 Bar = 0 2 mm

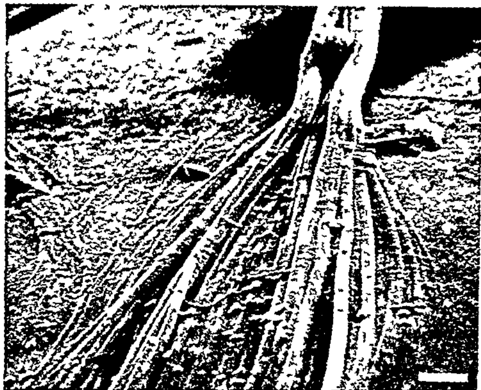


Fig 5
Detail of brush like ramification of a zonule inserting on the posterior lens surface × 1150 Bar = 10 μm

equator (Fig 6) The whole equatorial region of the lens is striated in a meridional direction (Fig 7)

In Fig 1 it is seen that a few zonule like strands attach to the posterior surface of the iris These strands are irregularly distributed along the corona ciliaris and their number is about half that of the ciliary processes Thin strands running in an oblique or circular direction may connect neighboring zonules (Figs 1 and 6) In high magnification a cobweb like veil can be seen in the ramifications of zonules or at their insertions on the lens By this method of preparation no matrix enveloping the zonules can be recognized

The main zonules do not form a cross when bridging between the lens and the ciliary body (Fig 6)

In some specimens all the fibrils running along the posterior lens surface end abruptly along a sharp continuous circular line obviously by being torn (Fig 11) This line is probably Egger s line to which the vitreous has been attached When

lium With special regard to the organization and insertion of the zonular fibrils *Invest Ophthal 10* 851–869
Rohen J W & Rentsch F J (1969) Der konstruktive Bau des Zonulaapparates beim Menschen und dessen funktionelle Bedeutung Morphologische Grundlagen fur eine neue Akkomodationstheorie *Albrecht v Graefes Arch Ophthal 178* 1–19
Vail D (1957) The zonule of Zinn and ligament of Wieger *Trans Ophthal Soc U K* 77 441–499

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in distinguishing between true zonules and the hyaloideo capsular fibrils of the ligament of Wieger inherent in other preparatory techniques e g embedding and sectioning

According to the findings in the present study the zonules are distinct real and independent entities which can not be considered as a part of the vitreous Their course is direct and simple The zonules bridge the shortest distance be tween the lens equatorial region and the ciliary body Here they insert on the anterior part of the ridges of the ciliary processes There is no systematic cross ing of zonules as they span between the lens and the ciliary processes The zonules do not run continuously from the lens toward ora serrata or pars plana ciliaris In the valleys between the ciliary processes there are no true zonules Zonule like strands which do not insert on the lens run in a meridional direction between the posterior end of the processes and pars plana and along the surface of pars plana They seem to reinforce these structures for forces in a meridional direction The forces involved in the mechanism of accomodation are thought to be transferred to the lens via these strands and the true zonules

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Raviola Giuseppina (1971) The fine structure of the ciliary zonule and ciliary epithe

tension glaucoma (Ehlers & Kruse Hansen 1974) These findings suggested that the corneal thickness influenced the reading obtained by applanation tonometry In the calibration of the applanation tonometer Goldmann assumed a corneal thickness of 0.5 mm and emphasized that theoretically the corneal thickness would influence the reading (Goldmann & Schmidt 1957) However at that time there were no data available on the variation of corneal thickness In the present report experimental data on the influence of corneal thickness and radius upon the applanation tonometer reading are reported for rabbit and man The findings were presented at the 15th Meeting of the Association for Eye Research Wurzburg October 1974

Material and Methods

Rabbit experiments Under intravenous barbiturate anaesthesia a canula was passed through the anterior chamber in rabbits weighing between 0 5 and 3 0 kg Openings in the canula permitted access to the aqueous The canula was connected to an adjustable saline reservoir and a Statham model P 23 pressure transducer (volume displacement 0 04 μl/100 mmHg) The transducer fed an electronic manometer (Ellab Blood Pressure Monitor M4 BCM) directly calibrated by water columns The rabbit was suspended in the supine position and the head placed in a Haag Streit slit lamp Corneal thickness was measured as previously

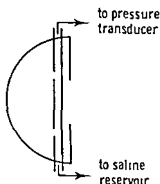


Fig 1
...stration of the placement of the canula in the anterior chamber of the rabbit

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APPLANATION TONOMETRY AND CENTRAL CORNEAL THICKNESS

BY

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Readings with the Goldmann applanation tonometer were made at various intraocular hydrostatic pressures and compared with central corneal thickness and radius in rabbit and in man Linear correlations were established between hydrostatic pressure and applanation readings with correlation coefficients close to 1 0 In rabbits the tonometer readings were generally too low In human eyes with a normal corneal thickness tonometer readings and hydrostatic pressure coincided with thick corneas the readings were too high with thin corneas too low The correlation between corneal thickness and the error of applanation tonometry (ΔP) was statistically highly significant ($P < 0\,001$) No statistical correlation could be established between corneal radius and ΔP Multiple regression taking thickness as well as corneal radius into consideration revealed only slightly higher correlation coefficients It is concluded that the central corneal thickness is a parameter which should be taken into consideration when evaluating applanation tonometer readings A Table is presented showing the correction to be added to the applanation reading at differing corneal thickness

Key words applanation tonometry – central corneal thickness – corneal radius – intraocular pressure – glaucoma

In previous publications an increased central corneal thickness was demonstrated in monosymptomatic ocular hypertension (Kruse Hansen & Ehlers 1971 Ehlers Kruse Hansen & Aasved 1975) and a reduced central corneal thickness in low

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Presented at the 15th Meeting of the Association for Eye Research Wurzburg October 1974

tension glaucoma (Ehlers & Kruse Hansen 1974) These findings suggested that the corneal thickness influenced the reading obtained by applanation tonometry In the calibration of the applanation tonometer Goldmann assumed a corneal thickness of 0 5 mm and emphasized that theoretically the corneal thickness would influence the reading (Goldmann & Schmidt 1957) However at that time there were no data available on the variation of corneal thickness In the present report experimental data on the influence of corneal thickness and radius upon the applanation tonometer reading are reported for rabbit and man The findings were presented at the 15th Meeting of the Association for Eye Research Wurzburg October 1974

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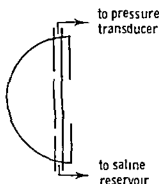


Fig 1
Diagrammatic illustration of the placement of the canula in the anterior chamber of the rabbit

reported (Ehlers & Kruse Hansen 1971) the pachometer being modified according to Mishima & Hedbys (1968) Intraocular tension was measured by an ordinary applanation tonometer and corneal radius by the Haag Streit keratometer

Human experiments Central corneal thickness and horizontal radius were measured with the above mentioned instruments Cases of astigmatism above 1 5 D were excluded After retrobulbar anaesthesia and prior to operation for cataract or glaucoma a small canula was passed into the anterior chamber under microscopical control The intraocular pressure was determined by the height of a saline reservoir above the eye and simultaneous applanation tonometry was performed with the Perkins or the Draeger handheld applanation tonometer calibrated against the standard Goldmann tonometer

Statistical treatment The data were evaluated by computor applying single and multiple linear regression models Significance levels were evaluated by the t distribution as the Kolmogoroff Smirnoff test showed distributions not incompatible with a Gaussian curve

Results

In the single rabbit and human eye a linear correlation was found between intraocular hydrostatic pressure and applanation tonometer reading In all experiments correlation coefficients above 0 96 were found

In the rabbit eyes the applanation readings were invariably too low The slope of the correlation lines increased with increasing corneal thickness (Fig 2) In rabbit eyes there is a positive correlation between corneal radius and thickness (Fig 3) which is not manifest in human eyes (Kruse Hansen 1971 Ehlers et al 1975) It could therefore be expected that data obtained from rabbit eyes would be of limited clinical interest and consequently it was decided to perform the subsequent experiments on human eyes

The linear correlation between intraocular hydrostatic pressure and tonometer reading for human eyes was much closer to proportionality than found for the rabbit The correlation coefficients approximated 1 and it was therefore decided to restrict the applanation tonometer readings to two different pressure levels in order to establish the correlation in the individual eye In 29 eyes with a normal cornea more especially without any signs of oedema three tonometer readings were taken at 10 mmHg and three at 30 mmHg intraocular hydrostatic

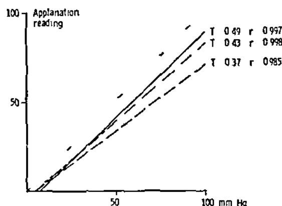


Fig 2

Three experiments on rabbits with different corneal thickness A linear correlation between applanation readings and intraocular pressures is verified by the high correlation coefficients (r) The slope of the lines increases with corneal thickness

pressure The differences between intraocular pressure and corresponding applanation reading ($\Delta P = P -$ appl reading) for each eye were compared statistically with corneal thickness and corneal radius

The results of the analysis are shown in Tables I and II The correlation matrix in Table I shows a strong linear dependence ($P < 0\ 001$) between ΔP_{10} and

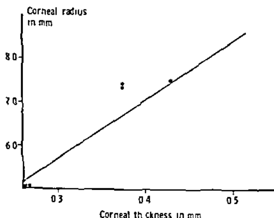


Fig 3

Correlation between corneal thickness and corneal radius in rabbits eyes Correlation coefficient 0 9?0 ($P < 0\ 001$) Regression equation R = 1 75 + 13.58 T A similar correlation is not found in human eyes

Table I
Correlation matrix

	Corneal radius	ΔP_{10}	ΔP_{30}
Corneal thickness	0 085	−0 707	−0 737
Corneal radius	–	0 079	0 050
ΔP_{10}	–	–	0 853

corneal thickness between ΔP_{30} and corneal thickness and between ΔP_{10} and ΔP_{30} No correlation exists between ΔP_{10} or ΔP_{30} and corneal radius nor between corneal thickness and corneal radius

Table II shows the four calculated regression equations The correlation coefficients are highly significant ($P < 0\ 001$) In the single regressions the regression coefficients are highly significant In the multiple regressions the partial regression coefficients with respect to corneal thickness are significant ($P < 0\ 001$) whereas the partial regression coefficients with respect to corneal radius are not

Table II
Single and multiple regression equations and correlation coefficient

Dependent	Variables	Correlation coefficients
ΔP_{10}	= 30 97 − 60 26 T t = 9 22 $P < 0\ 001$	r = 0 707 $P < 0\ 001$
ΔP_{30}	= 40 04 − 76 38 T t = 10 04 $P < 0\ 001$	r = 0 737 $P < 0\ 001$
ΔP_{10}	= 25 11 − 61 28 T + 0 82 R t = 9 48 t = 1 85 $P < 0\ 001$ $P < 0\ 06$	r = 0 721 $P < 0\ 001$
ΔP_{30}	= 34 25 − 77 39 T + 0 81 R t = 10 23 t = 1 56 $P < 0\ 001$ $P < 0\ 11$	r = 0 745 $P < 0\ 001$

T = corneal thickness R = corneal radius

statistically significant ($P > 0.05$) The differences between the y intercepts and between the regression coefficients are not statistically significant

It appears from this statistical evaluation that for an adequate description of the correlations it is sufficient to express ΔP_{10} and ΔP_{30} as functions of corneal thickness alone

The experimentally demonstrated linear correlation between intraocular hydrostatic pressure (P) and applanation tonometer reading (Appl) can be written

$$\text{Appl} = a \cdot P + b$$

which may be rearranged as

$$P - \text{Appl} = (1 - a) \cdot P - b$$

and written

$$\Delta P = (1 - a) P - b$$

This last equation shows linearity between ΔP and P and it is therefore possible to calculate the ΔP of any intermediate pressure level from the ΔP_{10} and ΔP_{30} by linear interpolation.

ΔP_{20} the error of the applanation reading at the upper limit of normal intra ocular pressure is a figure of clinical interest and is illustrated in Fig 4 Table

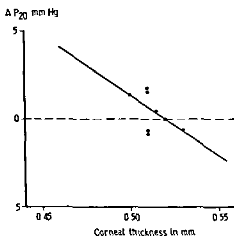


Fig 4

The error of the applanation tonometer reading at 20 mmHg (ΔP_{20}) is correlated to central corneal thickness Correlation coefficient 0.768 ($P < 0.001$) Regression equation $\Delta P_{20} = 35.51 - 68.33 \cdot T$ Regression coefficient highly significant ($t = 6.23$ $P < 0.001$) Including corneal radius the equation becomes $\Delta P_{20} = 29.69 - 69.34 \cdot T + 0.89 \cdot R$ The correlation coefficient is 0.779 Partial regression with respect to T is highly significant ($t = 6.37$ $P < 0.001$) partial regression with respect to corneal radius is not significant ($t = 1.08$ $P <$ [illegible])

Table III
Additive correction (ΔP) for Goldmann applanation tonometer readings

T	Appl				
	10	15	20	25	30
0.450	4.2	4.7	5.2	5.7	6.2
0.460	3.5	4.0	4.4	4.8	5.3
0.470	2.9	3.3	3.7	4.1	4.5
0.480	2.2	2.6	2.9	3.3	3.6
0.490	1.5	1.8	2.2	2.5	2.8
0.500	0.9	1.2	1.4	1.7	1.9
0.510	0.3	0.5	0.7	0.9	1.1
0.520	-0.4	-0.2	0.0	0.1	0.3
0.530	-1.0	-0.8	-0.7	-0.6	-0.5
0.540	-1.6	-1.5	-1.4	-1.3	-1.2
0.550	-2.2	-2.1	-2.1	-2.0	-2.0
0.560	-2.8	-2.8	-2.8	-2.8	-2.7
0.570	-3.4	-3.4	-3.4	-3.4	-3.4
0.580	-3.9	-4.0	-4.1	-4.1	-4.2
0.590	-4.5	-4.6	-4.7	-4.8	-4.9

The Table gives the correction to be added to the tonometer reading in order to obtain the intraocular hydrostatic pressure in mmHg T is thickness of cornea Appl is tonometer reading The Table can be applied to normal corneas

III shows the graphically obtained corrections which should be added to the applanation reading at any thickness and applanation level in order to obtain the intraocular hydrostatic pressure It is seen from Table III and from Fig 4 that for a normal corneal thickness the applanation tonometer reading coincides with the intraocular pressure However for a thin cornea the tonometer reading is too low and for a thick cornea it is too high

It will also be seen from Table III that the difference between the corrections at 30 mmHg and 10 mmHg varies with corneal thickness The smallest difference is not found as might be expected at the normal corneal thickness of 0.520 but at a value of about 0.56 The slope of the line connecting applanation reading to intraocular pressure is

$$a = 1 - \frac{\Delta P_{30} - \Delta P_{10}}{20}$$

The smaller the difference between ΔP_{30} and ΔP_{10} the more the slope approximates 1 The slope increases with corneal thickness (Fig 5) as was also found in rabbits (Fig 2)

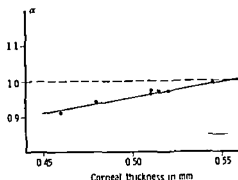


Fig 5

The slope of the lines connecting applanation readings and intraocular pressures ($a = 1 - \frac{\Delta P_{30} - \Delta P_{10}}{20}$) is probably correlated to corneal thickness Correlation coefficient 0.550 ($P \sim 0\ 05$) Regression equation $a = 0\ 561 + 0\ 781$ T

Discussion

In rabbits as well as in man linear correlations are found between intraocular pressures and applanation tonometer readings of the *in vivo* eye demonstrating the applicability to the eye of the Imbert Fick law for applanation of a sphere of infinitely small thickness

$$P = \frac{W}{A}$$

(P = pressure in sphere W = applanating force and A = applanated area) It is not surprising that the calibration of the Goldmann applanation tonometer intended for human eyes does not apply to rabbit eyes The readings were in variably too low although with increasing thickness the error (ΔP) decreased In human eyes a closer correlation was found between intraocular pressure and applanation reading

A statistically highly significant linear correlation was found between ΔP and the central corneal thickness ($P < 0\ 001$) No correlation could be demonstrated between ΔP and corneal radius and by multiple regression the correlation coefficient taking thickness as well as radius into consideration was only slightly higher than for thickness alone It therefore appears that the ΔP can be described as a function of corneal thickness

An objection to the experimental procedures could be the use of an "open system in which the human eye is directly connected to the reservoir An error could be introduced for instance if scleral rigidity was after all of importance for applanation tonometry The rabbit experiments in which the pressure was measured by a transducer of small volume displacement did not however lend any support to the importance of this error which moreover can hardly be correlated to corneal thickness

The importance of the corneal thickness for assessing the reliability of the applanation readings is illustrated in Fig 4 and in Table III It is seen that with a normal corneal thickness of about 0 520 the Goldmann applanation tonometer gives a correct value for the pressure When the thickness is below normal the applanation reading *is too low* and *when the thickness is above normal the* reading is too high This tendency was predicted by Goldmann but the magnitude of the error which may reach up to ± 5 mmHg within the normal range of corneal thickness (0 46–0 58 mm) *is surprising* It should be noted that the given *corrections are based on a series of 29 eyes* A larger series may well cause minor alterations in the figures

The experimental data reported in this paper stress the importance of the corneal thickness in the evaluation of glaucoma patients (Kruse Hansen & Ehlers 1971 Ehlers & Kruse Hansen 1974 Ehlers et al 1975) It would seem possible to explain at least some cases of low tension glaucoma and of ocular hypertension on the basis of an error of measurement caused by the abnormal corneal thickness

Applanation tonometry on oedematous corneas are at present being studied On a swollen cornea the applanation reading may be much too low It therefore seems necessary to distinguish between a normal thickness made up of collagen fibrils and a swollen thickness made up of interfibrillar aqueous solution

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RETINOCHOROIDAL VASCULAR ANASTOMOSIS IN TOXOPLASMIC CHORIORETINITIS

Report of a Case

BY

M SAARI R MIETTINEN H NIEMINEN and S RÄISÄNEN*

A clinical study of a case of toxoplasmic chorioretinitis was made with colour photographs and fluorescein angiography of the fundus during two recurrences The patient had toxoplasmic chorioretinal scars in both eyes A retinochoroidal vascular shunt was demonstrated in the perimacular scar of the left eye showing a recurrent satelite lesion adjacent to the macula at the age of 20 and a second recurrence 5 years and 3 months later To prevent a possible new recurrence from destroying the macular area a laser coagulation barrier was made between the chorioretinal lesion and the macula

Key words retina choroid – chorioretinitis – fluorescein angiography – laser coagulation – retinochoroidal vascular anastomosis – toxoplasmosis

An unusual form of new vessel formation a retinochoroidal vascular anastomosis has been demonstrated earlier in a presumed toxoplasmic retinochoroidal scar with fluorescein angiography (Gass 1970) and histologically (Kennedy & Wise 1971) To throw more light on the pathomechanism of toxoplasmic chorioretinitis we describe here a case where a venous retinochoroidal anastomosis occurred in a chorioretinal scar which revealed a satelite recurrence twice and which showed positive serologic tests for toxoplasmosis

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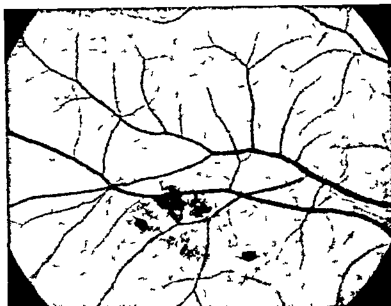


Fig 1

Right fundus Old toxoplasmic chorioretinal lesions (thick arrows) near inferior temporal vessels Remnants of perivasculitis as irregular vascular sheathing around the arterioles and veins (thin arrows)



Fig 2

Left fundus a) recurrent fresh satelite lesion adjacent to the inferior margin of old scar b) healed and pigmented satelite lesion 16 months later

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RETINOCHOROIDAL VASCULAR ANASTOMOSIS IN TOXOPLASMIC CHORIORETINITIS

Report of a Case

BY

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A clinical study of a case of toxoplasmic chorioretinitis was made with colour photographs and fluorescein angiography of the fundus during two recurrences The patient had toxoplasmic chorioretinal scars in both eyes A retinochoroidal vascular shunt was demonstrated in the perimacular scar of the left eye showing a recurrent satelite lesion adjacent to the macula at the age of 20 and a second recurrence 5 years and 3 months later To prevent a possible new recurrence from destroying the macular area a laser coagulation barrier was made between the chorioretinal lesion and the macula.

Key words retina choroid – chorioretinitis – fluorescein angiography – laser coagulation – retinochoroidal vascular anastomosis – toxoplasmosis

An unusual form of new vessel formation a retinochoroidal vascular anastomosis has been demonstrated earlier in a presumed toxoplasmic retinochoroidal scar with fluorescein angiography (Gass 1970) and histologically (Kennedy & Wise 1971) To throw more light on the pathomechanism of toxoplasmic chorioretinitis we describe here a case where a venous retinochoroidal anastomosis occurred in a chorioretinal scar which revealed a satelite recurrence twice and which showed positive serologic tests for toxoplasmosis

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anterior chamber but the vitreous was hazy On the temporal side of the macula the fundus revealed an old chorioretinal scar with heavily pigmented and sharply demarcated margins In the centre of the scar the retina and choriocapillaris were destroyed and only three blood filled choroidal vessels were observed against the bare sclera At the temporal margin of the scar a venous chorioretinal anastomosis was seen and adjacent to the inferior margin of the scar there was a fresh satelite lesion with localized oedema (Fig 2 a) There was also retinal periphlebitis with sheathing of the vessels

The condition improved over the next 2 months When the patient was seen 16 months later both eyes were quiet and the satelite lesion of the left eye was pigmented (Fig 2 b)

In May 19 4 the patient noticed blurred vision and spots in front of the left eye Ophthalmic examination in the right eye revealed visual acuity 1 2 the eye was quiet and the old lesions were seen in the fundus near the inferior temporal vessels Visual acuity in the left eye was 1 0 Auto plot Tangent Screen (Bausch & Lomb) demonstrated a wide paracentral scotoma Slight flare and few cells were seen on slit lamp examination The vitreous was very hazy with some dense opacities Close to the inferior edge of the healed satelite lesion there was a new active satelite lesion Around the retinal vessels there was secondary retinal periphlebitis with sheathing of the vessels

In fluorescein angiography the margins of the chorioretinal scar had begun to fluoresce in the early filling phase of the choroid The atrophic centre of the scar was non fluorescent despite three large choroidal vessels The fresh active satelite lesion showed no fluorescence in the phase of choroidal circulation It became visible first after several



Fig 4

Fluorescein angiography showing the retinochoroidal anastomosis a) retinal arteriovenous phase Retinochoroidal anastomosis (arrow) Thinner central part of the vein starting from nasal edge of scar (thick arrow) Thin neo vascular capillaries (thin arrows) against non fluorescent centre of scar Pseudofluorescence eliminated with correct combination of filters b) late venous phase The retinal vein is normal peripherally to the retinochoroidal anastomosis

Case Report

A 25 year old man who after an injury to the root of the nose at the age of 20 in January 1969 noticed black areas following the eye movements in his left eye Ophthalmic examination in the right eye revealed visual acuity 1 4 the eye was quiet but the inferior temporal periphery of the fundus showed pigmented choroidal scars (Fig 1) The left eye had visual acuity 1 0 there was no flare and no cells were seen in the

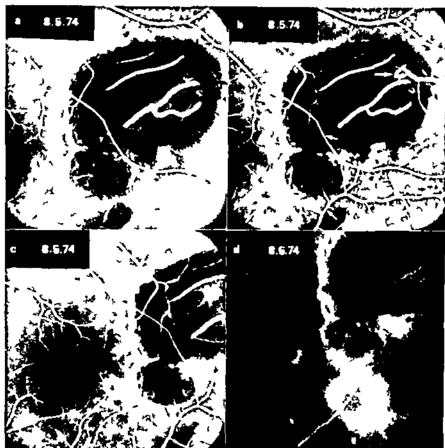


Fig 3

Fluorescein angiography from the left fundus a) arteriolar phase Margins of the scar fluoresce together with choroidal fluorescence In the centre of the scar fluorescence is absent despite three large choroidal vessels b) retinal arterio venous phase Retino choroidal anastomosis (thick arrow) Retinal arteriole crossing lower edge of the old lesion and retinal vein crossing the non fluorescent fresh satellite lesion are narrowed (thin arrows) c) venous phase Locality of the lesion adjacent to macula is seen Developing fluorescence (thin arrows) from margins of non fluorescent fresh satellite lesion d) late phase Fresh satellite lesion stains diffusely

The only significant findings during the two recurrences from general examination X ray and laboratory studies were positive serological test titers for toxoplasma At the beginning of the first recurrence the toxoplasmosis dye test (DT) and complement fixation test (CF) were negative and 3 weeks later both were 1 4 positive on a repeat test 18 months later DT was 1 4 positive and CF negative During the second recidive CF was 1 8 positive (the control serum was MY 10/23/67 from Milford N Lunde Laboratory of Parasitic Diseases National Institute of Allergy and Infectious Diseases Bethesda Maryland) indirect fluorescent antibody test (IFA) 1 256 positive (the control serum used was from Milford N Lunde N o 618/73) and the indirect fluorescent anti body test for the demonstration of immunoglobulin G toxoplasma antibody (IgG IFA) was 1 128 positive (the control serum was from Milford N Lunde N o 113/74) and IgM IFA was negative (below 1 10)

Discussion

The present case revealed bilateral chorioretinal scars with two recurrences of the paracentral lesion in the left eye Bilateralism of involvement centrality of location and a history of recurrence all suggest congenital toxoplasmic infection (Fair 1961) The first recurrence of the present case appeared at the age of 20 and the second 5 years and 3 months later The recurrence usually appears be tween 10 and 30 years of life and intervals between recurrences thereafter vary from a few months to several years (Fair 1961)

Dye test titers in established ocular toxoplasmosis can be low even in the face of exacerbations (Zscheile 1964) It is becoming agreed that a positive serologic test for toxoplasmosis by any of the accepted methods is significant at any titer provided the patient has a morphologically compatible fundal lesion (Scott 1974) and other causes of focal retinochoroiditis for example lues have been excluded (O Connor 1974) In the present case at the beginning of the first recurrence there were negative (1 4) DT and CF tests for toxoplasmosis but a repeat test 2 weeks later showed low positive DT and CF titers During the second recurrence IFA 1 256 and IgG IFA 1 128 were positive while IgM IFA was negative which supports our opinion of an old possibly congenital toxoplasmic chorio retinitis with two recurrences and according to Remington et al (1968) for example excludes the diagnosis of acquired acute toxoplasmosis

In a few cases of ocular toxoplasmosis a history of trauma prior to the onset of the recurrence is obtained (Duke Elder & Perkins 1966) as was found in the present case during the first recurrence However blunt local trauma did not produce recurrence of experimental toxoplasmic chorioretinitis in rabbits (Nozik & O Connor 1970)

Ocular toxoplasmosis most often causes a focal exudative necrotizing retino choroiditis with destruction of a segmental area of the retina Bruch s membrane and the choriocapillaris Perivascular cuffs of inflammation surround retinal vessels which at the area of active lesion are narrowed as was seen in the pre sent case (Fig 3) Extension of the perivascular infiltration forming a thrombus in the lumen of the vein and the local inflammation with intense congestion oedema and necrosis may cause the retinal vein which crosses the lesion to become obliterated and destroyed Venous occlusion produces stasis capillary dilatation aneurysm formation and vasoproliferation These vascular changes may result in formation of the rare retinochoroidal anastomosis at the site of the lesion as seen in the present case or even a racemose angioma of the retina (Stucchi et al 1972)

If the chorioretinal lesion does not involve the macula central vision may be normal Paracentral scars may recur and go on to involve the macular area making good central vision impossible To prevent this from happening in the present case where already two paracentral recurrences were seen a fire break laser barrier (Spalter et al 1968) was made between the macula and the lesion

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THE EFFECT OF THE ANTERIOR AND POSTERIOR PARTS OF THE EYE ON ULTRASONIC INTENSITY

BY

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The effect of the anterior and posterior parts of a pig eye and the halves of a plastic ball on relative sound intensities was examined using a steel ball with a diameter of 2 mm as the reflector Six acoustic cross sections of the halves of the pig eyes were examined Intensity measurements were performed 1 mm from one another in each acoustic section The lens caused a round relative acoustic shadow with a diameter of 5-7 mm which attenuated ultrasound up to 15–20 dB The sclera caused, 3–4 mm from the edges either an absolute or an almost absolute shadow which was circular in shape and had a diameter of 1-2 mm The edges of the sclera caused a slight increase in sound intensity which was probably due to their focusing effect A narrow circular acoustic shadow similar in shape to the above mentioned but relative was found when the effect of the halves of the plastic ball was examined The edges of the halves of the plastic ball did not have any focusing effect

Key words ultrasonography – ultrasonic intensity – acoustic shadow – acoustic cross section – pig eye – sclera – anterior part – posterior part

In earlier examinations refraction and reflection up to total reflection were observed when the sound beam hit the surface of the eye in a slanting direction

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(Oksala & Hakkinen 1969 Oksala & Gerstner 1972) When the effect of the whole eye on sound intensity was examined in several accoustic cross sections it was observed that the lens also greatly attenuated ultrasound causing a round relative acoustic shadow and that a narrow circular absolute or almost absolute acoustic shadow appeared when the sound beam hit the anterior part of the sclera at a distance of approx 3–4 mm from its equator (Oksala 1973) The conclusion was reached from experimental measurements that the sclera some times slightly focused ultrasound when the sound beam hit the anterior part of the sclera near its equator (Oksala & Niiranen 1970 Oksala & Gerstner 1972)

The aim of this study has been to try to discover what effect the anterior and posterior parts of the eye respectively have on ultrasonic intensity

Material and Methods

The experiments were performed on recently slaughtered pig eyes all of which were approximately the same size (23–25 mm) The eyes were prepared by re moving all external parts after which they were divided along the equator so that the anterior uvea the lens and the anterior part of the vitreous were left in the anterior part of the eye whereas all tissues inside the sclera were removed from the posterior part The effect of the convex and concave parts of a plastic ball (diameter 38 mm) on the sound intensity was also examined in order to find out if ultrasound acts in the same way in any equally shaped materia as it does in the eye shell

The ultrasound equipment was Kretztechnik s model 7000 and it was connected to a Tektronix Oscilloscope 515 for measurements When investigating pig eyes an unfocused transducer 7 5 MHz/2 mm was used thus having a near field of approx 5 mm To examine the effect of both halves of the plastic ball on ultra sonic intensity an unfocused transducer 6 MHz/5 mm with a near field of ap prox 25 mm was used

The transducer was placed on a stand in a bowl filled with distilled water An SKF steel ball with a diameter of 2 mm was placed under the transducer at a fixed distance from it in the acoustic centre of the sound field A thin copper wire stand was placed between the transducer and the steel ball The halves of the eye and the plastic ball could be moved along this stand at a fixed distance from the transducer and the steel ball When the halves of the eye and the plastic ball were moved between the transducer and steel ball which

were both stationary the desired acoustic cross sections could be made The longest cross section went through the centre of the halves of the eye and the plastic ball The shortest cross sections were obtained from the edges of the hal ves of the eye and the plastic ball

The distance between the transducer and the steel ball was the same through out the examinations i e 30 mm When the effect of the anterior part of the pig eye on sound intensity was examined the shortest distance from the transducer to the centre of the cornea was 5 mm In examinations carried out on the effect of the posterior part of the pig eye on sound intensity the shortest distance be tween the sclera and the steel ball was 5 mm. When the effect of both halves of the plastic ball was examined the distance from the transducer to the steel ball was 45 mm the shortest distance from the transducer to the centre of the convex being 5 mm and that between the centre of the concave and the steel ball also 5 mm

Two different degrees of amplification in the equipment (dB reserves 20 and 2) were used in examinations on the effect of both halves of the pig eyes The stronger amplification was used when a strong acoustic shadow caused by the lens or the eye shell was observed This was to find out whether the shadow was absolute or relative when using this equipment The differences in dB be tween the amplifications were taken into consideration when presenting the final results and the following results can be regarded as having been obtained with the same amplification.

The measurements were performed in six acoustic cross sections when examin ing the effect of both halves of the pig eyes on the sound intensity In each cross section the echo amplitudes reflected by the steel ball were measured 1 mm from one another Thus about 24 amplitude measurements were performed in the longest acoustic cross section The measurements at a distance of 2 mm from one another were made in five acoustic cross sections when the effect of both halves of the plastic ball was examined Each acoustic cross section gave echo amplitude curves in which the amplitudes represented relative intensities

Twelve pig eyes were examined and the measurements concerning the effect of both the convex and concave halves of the plastic ball were performed five times

Results

When the effect of the halves of the eye and the halves of the plastic ball on ultrasonic intensity was examined almost the same echo amplitudes reflected by

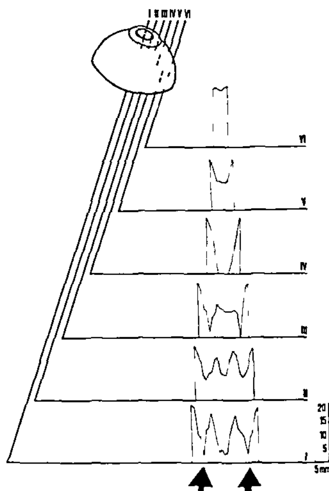


Fig 1
The effect of the anterior part of the eye on sound intensity in six acoustic cross sections X axis is given in millimeters y axis presents relative echo amplitudes

the steel ball were received in each acoustic cross section and consequently the results were easily repeatable Because of this only one amplitude curve is presented from the measurements made in each acoustic cross section of the pig eyes

Fig 1 shows the effect of the anterior part of the eye on ultrasonic intensity

Amplitude curve I represents the variations in amplitudes and consequently also in sound intensity at a situation where sound has travelled in an axial direction through the anterior part of the eye via its centre Three relative acoustic shadows are presented in curve I The shadow caused by the lens is seen in the middle and at a distance of 3–4 mm from both edges of the sclera there is a narrow relative shadow which is probably due to reflection and refraction of ultrasound in the anterior part of the sclera (arrows) In curve II sound has travelled through the edges of the lens and consequently the relative shadow caused by the lens is shorter and weaker than in curve I Curve III was obtained when sound passed the lens but travelled through the edges of the cornea The acoustic shadow at one side of the curve caused by the edges of the sclera is absolute

Curve IV was obtained when the cross section passed through the sclera at a distance of 3–4 mm from its edges In the middle there is a 4 mm long absolute shadow Curve V represents the measurement results in a cross section 2–3 mm from the edge of the sclera and here a 4 mm long rather weak relative shadow can be observed Curve VI represents the measurement results of the last acoustic cross section which travels near the edge of the sclera The amplitudes which were measured were all approximately the same height the relative value being about 20

The amplitude reading was 16 when the measurements were performed in water alone All six amplitude curves showed that the amplitude reading was 18–20 when sound travelled through the edges of the sclera

Fig 2 shows the amplitude curves in six different cross sections when sound has travelled through the rear wall calotte formed by the sclera Amplitude curve I was obtained when the acoustic cross section travelled through the centre of the rear wall calotte Relative acoustic shadows caused by the edges of the sclera can be seen Curve II greatly resembles the above mentioned curve In curve III in addition to the absolute shadow caused by the edges of the sclera a lowering in intensity caused by the central parts of the sclera can also be seen The conic shape of the central parts is noticeable in curves I II and III In curve IV the sclera has caused a strong approx 8 mm long acoustic shadow In curve V the central shadows caused by the sclera is weaker and shorter than the above mentioned shadow Curve VI was obtained when the sound beam had travelled right through the edges of the sclera The length of the curve is only 4 mm and the amplitudes measured are almost of the same height i e with a relative degree of 18

The curves in Fig 2 also show that when sound has travelled through the edges of the sclera higher amplitudes and consequently also higher sound intensities can be measured in measurements performed in just water

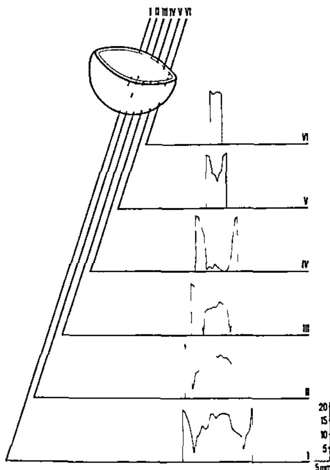


Fig 2
The effect of the posterior part of the eye on sound intensity in six acoustic cross sections

In examining the effect of the convex and concave parts of the plastic ball on sound intensity clear narrow relative shadows which according to their location corresponded to the shadows caused by the edges of the sclera were observed in measurements performed approx 5 mm from the edges There is a similar conic shape as seen in Fig 2 in these midmost amplitude curves How ever no increase in sound intensity could be measured when sound had travelled through the edges of the plastic ball

Comments

There were three different kinds of effect of the anterior part of the pig eye on sound intensity The lens caused a round relative acoustic shadow with a diameter of 5-7 mm in which attenuation was at its strongest about 15-18 dB compared to attenuation in water When the sound travelled parallel to the axis of the eye and hit the sclera at an approximate distance of 3-4 mm from its edge either an absolute or an almost absolute acoustic shadow shaped like a thin circle and with a diameter of approx 1-2 mm could be observed with the equipment that was used When sound travelled through the sclera near its edge intensities 10-20 % stronger than in the control measurements made in water could be measured

The concave calotte changed the sound intensity in three different ways when sound travelled through the rear wall formed by the sclera An absolute, or an almost absolute acoustic shadow with a diameter of approx 1-2 mm forming a narrow circular shadow in regard to the whole half of the eye was measured when sound travelled through the concave surface of the sclera approx 3-4 mm from the edge of the calotte When sound travelled through the edges of the sclera intensities which were approx 10-15 % higher than those measured in just water could be measured in the sound field The conic shape in the middle of the amplitude curves can be regarded as due to the increase in attenuation caused by reflection and refraction in the sclera when moving from the centre towards the edges of the calotte

In examinations where dead materia or halves of a plastic ball with a very thin shell (0 5 mm) were used it could be observed when measuring the sound field that a strong relative acoustic shadow always appeared after sound had travelled through the halves of the ball at a distance of approx 5 mm from its edges The acoustic shadow was in this case circular and had a diameter of only 1-2 mm On the other hand the edges of the halves of the plastic ball did not cause any increase in sound intensity It is possible that the shell of the plastic ball was not thick enough to cause a focusing effect on sound

If the lens is not taken into consideration both halves of the eye had a very similar effect on sound intensity This is very logical since the anterior and posterior parts of the eye resemble in shape the halves of a ball where again equal reflection and refraction occur if the angles of incidence are the same in both halves The possible slight focusing effect of sound when it has travelled through the edges of the sclera is interesting theoretically but at least for the time being it has only a very limited clinical significance

Also by using halves of a plastic ball in examinations it was possible to show that when sound meets the eye shell it follows the same physical laws as in any materia of similar shape

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THE EFFECT OF CITRATE AND COAGULATED BLOOD ON ULTRASONIC INTENSITY

BY

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Pig eyes were used in the examination The rear wall calotte formed by the sclera was filled alternately with citrate and coagulated blood The effect of these on sound intensity was investigated by measuring relative sound intensities in six acoustic cross sections 1 mm from one another The eye shell i e the sclera had a strong effect on the echo amplitudes in the three outermost acoustic cross sections In the three cross sections obtained from the centre of the calotte it was observed that coagulated blood attenuated ultrasound 10–15 dB more than citrate blood In the centre of the calotte attenuation was caused by coagulated blood but the effect of reflection and refraction in the sclera increased towards the edges As far as clinical research is concerned it can be concluded that when a large blood coagulation fills the vitreous the tissues behind it have to be examined with a greater sound energy than when the examination is performed through a clear vitreous or one filled with exudate

Key words ultrasonography – ultrasonic intensity – acoustic shadow – acoustic cross section – pig eye – sclera – rear wall calotte – citrate blood – coagulated blood

In an earlier experimental study (Oksala & Salminen 1967) there was an absorption difference of approx 10 dB between a 12 mm high column of water and coagulated blood of the same thickness measured with frequencies of 6 MHz and

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10 MHz A pig sclera acted as the reflector of sound Oksala (1973) established that water and citrate blood attenuated ultrasound almost equally as much with frequencies of 6 7 1/2 and 10 MHz whereas coagulated blood attenuated clearly more ultrasound This difference in attenuation was strongest i e 10–15 dB in a 12 mm high column with a frequency of 10 MHz In this study the scleral calotte of a pig eye was filled with the blood to be examined and the sound was directed perpendicularly to the rear wall A 1 5 mm steel ball acted as the reflector

During the above mentioned investigations relative intensity measurements were performed by examining one single place both in the citrate and coagulated blood Then it was not possible to obtain information about the effect of both the rear wall calotte and the investigated material on sound intensity in the whole area of the calotte

In this study both comparisons were made on the effect of citrate and coagulated blood on ultrasonic intensity and at the same time examinations were made on the effect of these two combined with the scleral calotte

Material and Methods

The experiments were performed on recently slaughtered pig eyes all of which were of the same size (diameter 23–25 mm) All external parts were removed and only the calotte formed by the scleral rear wall was used

The ultrasonic equipment was Model 7000 manufactured by Kretztechnik (Zipf/Austria) and it was connected to a Tektronix Oscilloscope 515 for measurements A 7 5 MHz/2 mm transducer having a near field of approx 5 mm was used

The transducer was placed on a stand in a bowl filled with distilled water Under the transducer in the centre of the acoustic field there was an SKF steel ball with a diameter of 2 mm The rear wall calotte made by the sclera was placed on a copper wire stand where it was possible to move the calotte in such a way that when the transducer and the ball were stationary acoustic cross sections could be taken from any point of the calotte one wished The distance of the transducer from the steel ball was 30 mm and the shortest distance from the bottom of the calotte to the steel ball was 5 mm

First the sound field of the transducer was measured in just water After that the scleral calotte was placed on a stand and filled with citrate blood The first

cross section went through the centre of the calotte and the five following ones in the same direction as the first but about 2 mm from one another Thus the length of the longest cross section was about 23–25 mm and that of the shortest which was obtained from the edge of the calotte about 5 mm The echo amplitudes reflected by the steel ball were examined in each acoustic cross section 1 mm from one another These amplitudes represented relative sound intensities After this the calotte was filled with coagulated blood and the same measurements were performed

Ten different calottes were used in measurements of both citrate and coagulated blood Two different degrees of amplification in the equipment i e dB reserves 20 and 2 were used in the examinations With the stronger amplification i e dB reserve 2 the aim was to find out whether the acoustic shadows with this equipment were absolute or not In the final results the difference between these two amplifications in decibels was taken into consideration and the results were presented in such a way that they can be regarded as having been obtained with one and the same amplification

Results

Amplitude curves measured in the same acoustic cross sections were easily repeatable and because of their similarity only one of them is presented Fig 1 shows the effect of the rear wall calotte filled with citrate blood on sound intensity The amplitude curves represent relative sound intensities in each of the six cross sections In curves I II and III both inner surfaces of the sclera cause a clear relative acoustic shadow approx 3–4 mm from the edges (arrows) The curves are cone shaped in the middle In the centre in curve IV a 7 mm long absolute acoustic shadow could be observed with the equipment which was used In curve V there is in the centre a slight relative shadow caused by the sclera Curve VI is almost like a column and the amplitudes (18–20) are little higher than the highest amplitudes obtained in just water which always had the value of 16

Fig 2 represents relative sound intensities in six acoustic cross sections when the rear wall calotte was filled with coagulated blood The same acoustic shadows caused by the edges of the sclera as in Fig 1 are observed When comparing the amplitude curves in Figs 1 and 2 with each other distinct differences occur only in curves I II and III Curves IV V and VI are in both Figures

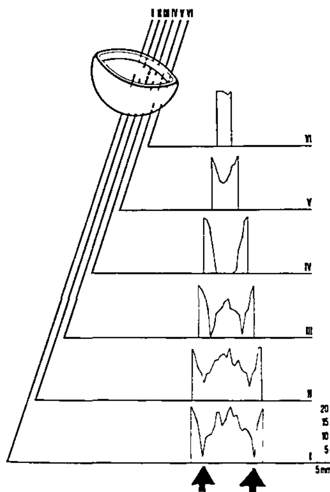


Fig 1
The effect of citrate blood and the rear wall calotte on sound intensity in six acoustic cross sections X axis is given in millimeters y axis presents relative echo amplitudes

almost similar to one another In Fig 2 the amplitudes representing intensities in curves I II and III are clearly lower than in the corresponding curves in Fig 1 In addition the amplitudes in curves I and II do not form a cone in the middle A 12 mm high column of coagulated blood when compared to citrate blood caused a 10–15 dB greater attenuation in the centre of the calotte

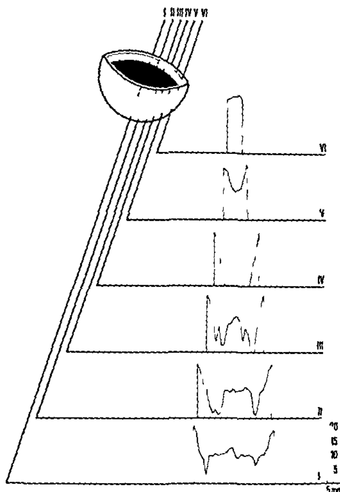


Fig 2
The effect of coagulated blood and the rear wall calotte on sound intensity in six acoustic cross sections

Comments

When relative sound intensities in acoustic cross sections were compared with one another using the above mentioned methods only the first three acoustic cross sections could be used As a matter of fact only the eye shell i e the sclera had an effect on the shapes of the amplitude curves of the three outermost acoustic cross sections The heights and shapes of these three outermost amplitude

curves corresponded completely with the results that have been obtained from an earlier study of the effect of the whole pig eye (Oksala 1973) and in a later one on the effect of both halve of the eye on sound intensity (Oksala 1975)

In Fig 1 the central part of amplitude curves I II and III representing the effect of citrate blood is cone shaped This might be due to the increasing re flection and refraction of sound when the angle of incidence of sound extends from the centre of the calotte towards the edges These results are exactly the same as observations which were made in a study on the effect of the rear wall calotte on sound intensity in just water (Oksala 1974)

Amplitude curves I II and III in Fig 2 represent the effect of coagulated blood on sound intensity They show that with the amplification used and at a distance of 12 mm coagulated blood attenuated ultrasound 10–12 dB more than the corresponding column of citrate blood This result corresponds to the figures obtained in earlier experimental examinations Amplitude curves I and II in Fig 2 as far as the effect of coagulated blood is concerned are very similar in height and no cone formation can be observed The similarity in amplitudes is perhaps due to the fact that attenuation in the centre of the calotte is caused by coagulated blood but in the edges by reflection and refraction of sound in the sclera Amplitude curve II in Fig 2 is on the other hand more irregular and shows that coagulated blood may contain parts having quite different acoustic effects

In Figs 1 and 2 in curve VI higher amplitudes than those measured in just water are observed This points to the fact that the edges of the sclera may have a focusing effect on sound (Oksala & Gerstner 1972)

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PROTEIN DIFFUSION BARRIERS IN THE ANTERIOR UVEA OF THE RABBIT EYE

BY

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The distribution in the anterior uvea of the protein tracer horseradish peroxidase has been studied in eyes of albino rabbits The tracer was injected intravenously 30 or 60 min before enucleation Light microscopy of frozen sections and of semithin sections of Epon embedded material revealed 1 The stroma of the iridial and ciliary processes and the choroid contained considerable amounts of peroxidase reaction product 2 In the iris and the ciliary body apart from the processes (the ciliary body base) the peroxidase reaction product was confined to the vessels 3 A sharp demarcation of peroxidase activity was observed between the iridial processes and the iris stroma and between the ciliary processes and the ciliary body base

By electron microscopy peroxidase reaction product was found in the vessels and the stroma of the ciliary processes In the ciliary body base reaction product was found within the vessels but not in the stroma The endothelium of these vessels had no fenestrations and there was no sign of passage of peroxidase across the endothelium either by the interendothelial clefts or by vesicular transport The study indicates that there is a functional barrier against diffusion of proteins from the iridial and ciliary processes across the iris and the ciliary body base

Key words ciliary body – iris – anterior chamber – aqueous humor – blood aqueous barrier – blood vessels – peroxidase – proteins – rabbit

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It has been known for some years that the extravascular tissues of the ciliary processes contain considerable amounts of serum proteins due to high permeability of the vessels (Bill 1964) In the aqueous humor only traces of proteins are present under normal conditions A diffusion barrier to proteins between the ciliary processes and the posterior chamber is located to the epithelium (Shiose 1970 Smith 1971 Vegge 1971 a)

However a functional barrier to movement of proteins from the ciliary and iridial processes into the aqueous humor across the ciliary body and iris must also exist As far as the authors are aware no morphological study on this barrier with the use of tracer technique has been reported

The purpose of the present work was to study the barrier between the ciliary and iridial processes and the anterior chamber In an attempt to demonstrate this functional barrier the movement of the protein tracer horseradish peroxidase in these tissues was studied This protein has a molecular weight of about 40 000 and an approximate diameter of 4 nm

Material and Methods

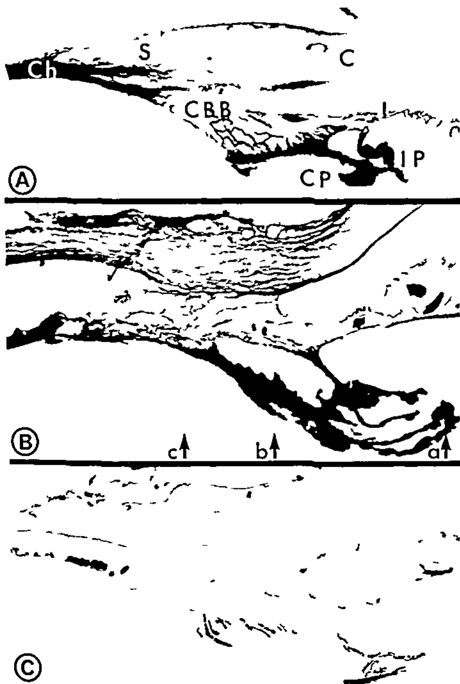
Eyes from three albino rabbits weighing about 3 kg were used Before experimentation all eyes were found normal determined with slit lamp and ophthalmoscopic examination

One rabbit was injected intravenously with 460 mg horseradish peroxidase (Sigma type II) dissolved in 5 ml Ringer solution and was killed after 30 min One rabbit was injected intravenously with 300 mg peroxidase and received an additional injection of 100 mg after 30 min This rabbit was killed 60 min after the first injection As control one rabbit did not receive peroxidase The

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Fig 1

Meridional frozen sections of rabbit eyes A Demonstration of peroxidase activity in an eye enucleated 30 min after intravenous injection of peroxidase Note intensive staining of iridial (IP) and ciliary processes (CP) and choroid (Ch) In the ciliary body base (CBB) and the iris (I) staining is confined almost exclusively to the vessels B This eye was enucleated 60 min after the first peroxidase injection In principle the distribution of the peroxidase reaction product is the same as in Fig 1 A except for some more staining of the sclera C Section from an eye of a rabbit that had not received peroxidase. The tissues were incubated as for peroxidase activity Endogenous staining within the vessels is due to peroxidase activity of the erythrocytes The arrows marked a b and c in Fig 1 B indicate the approximate locations of the transverse section shown in Figs 2 and 3 A B and C × 40


S
C
Ch
CBB
I
IP
CP
A
B
c
b
a
C

rabbits were killed with an overdose of sodium pentobarbital and the eyes were immediately enucleated They were opened broadly at the equator and immersed in 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 for 2 hrs During the prefixation the lens was removed from behind and the anterior segment was cut into large sectors The tissues were washed over night in 0 1 M phosphate buffer pH 7 4 containing 5 % sucrose

Frozen sections of 40 μm in thickness were cut meridionally with a freezing microtome These sections included cornea sclera iris ciliary body and the peripheral parts of retina and choroid Sections were also cut transversly to this through the iris and the ciliary body The sections were rinsed briefly in distilled water incubated in diaminobenzidine H_2O_2 solution (Karnovsky 1967) for about 30 min and rinsed again in distilled water Some sections were mounted on glass slides and examined directly with the light microscope Other sections were postfixed for 1½ hrs in 1 % OsO_4 in Millonig s phosphate buffer *dehydrated in graded series of ethanol treated with propylene oxide and em*bedded in Epon 812 For light microscopy 1 μm thick sections were cut from these blocks Ultrathin sections from the ciliary processes and the ciliary body base were cut with an LKB Ultratome and examined in a Siemens Elmiskop IA The sections were examined uncontrasted or after contrasting with an aqueous solution of uranyl acetate and alkaline lead

Results

In the following the terms iridial and ciliary processes are used in accordance with Kozart (1968) The ciliary body apart from the processes is termed the ciliary body base

Light microscopy

The distribution of the peroxidase reaction product in the different tissues of the anterior segment of the eye is shown in the light micrographs of frozen sections in Fig 1 Whether the eyes were enucleated 30 or 60 min after the peroxidase injection made only minor differences to the localization of peroxidase In sections from the rabbit that had not received peroxidase endogenous staining of the erythrocytes was found Apart from this no reaction product was observed (Fig 1 C)

Iris In the iris the peroxidase reaction product was confined to the vessels whereas the iris stroma was devoid of the product (Figs 1 2 A 3 A) The stroma of *the iridial processes was intensively stained At the base of these processes* there was a sharp demarcation between the stained iridial processes and the unstained iris stroma (Fig 3 A)

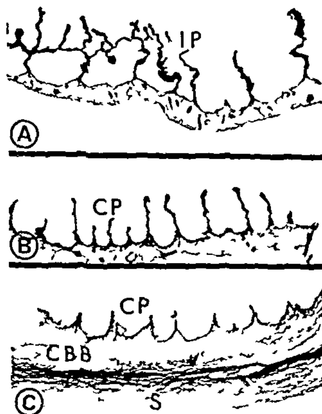


Fig 2

Frozen sections from an eye that was enucleated 60 min after the first peroxidase injection The sections were cut transversly to the meridional sections through the iris (A) and the anterior (B) and posterior parts (C) of the ciliary body The approximate locations of the sections are shown by arrows in Fig 1 B A Peroxidase reaction product is present in the iris vessels but not in the stroma The iridial processes (IP) are blackened by reaction product Iris (I) B and C The ciliary processes (CP) are blackened by peroxidase reaction product in contrast to the ciliary body base (CBB) Sclera (S) × 97

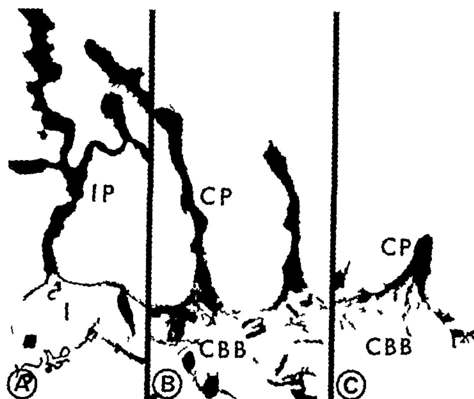


Fig 3

Higher magnifications of the sections shown in Fig 2 Note the sharp demarcation of the peroxidase activity between the iridial processes (IP) and the iris stroma (I) and between the ciliary processes (CP) and the ciliary body base (CBB) × 104

Ciliary body The ciliary processes were intensively stained in the same manner as those of the iris (*Figs 1 2 B C 3 B C 4*) Again there was a well defined demarcation between the ciliary processes and the ciliary body base where only a faint brown staining was observed The vessels of the ciliary body base were however blackened indicating a permeability barrier located in the vessel wall (Fig 4) In the ciliary body stroma adjacent to the anterior chamber angle no definite staining was observed (Figs 1 4)

Choroid The choroid was blackened (Fig 1) whereas no staining was found in the peripheral retina

Sclera and cornea The sclera overlying the choroid was markedly stained with decreasing intensity towards the outer surface A slight staining was found in

the sclera overlying the ciliary body base most marked at the border between these tissues (Fig 1) At the limbus there was a relatively heavy staining beneath the epithelium and in the Descemets membrane

Electron microscopy

The ciliary processes and the ciliary body base were studied by means of electron microscopy In the ciliary processes peroxidase reaction product was found in the lumen of the vessels in the stroma and around the basal (pigmented) epithelial cells (Fig 5 A) indicating that these vessels are permeable to peroxidase In the ciliary body base the vessel lumina contained peroxidase reaction product whereas this was not found outside the vessel walls (Fig 5 B C) Fenestrations

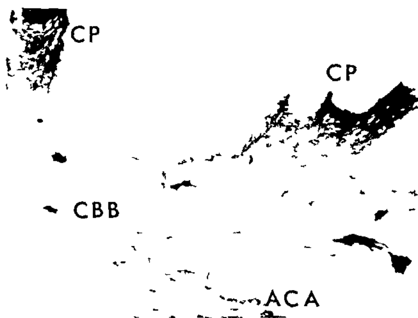


Fig 4

Unstained light micrograph of meridional section from tissue embedded in Epon Peroxidase reaction product is present in the vessels and the stroma of the ciliary processes (CP) In the rest of the ciliary body (CBB) reaction product is confined to the vessels The electron micrographs shown in Fig 5 B and C are from this region Anterior chamber angle (ACA) × 156



Fig 3

A Electron micrograph from a ciliary process Peroxidase reaction product is present in the vessel lumen (Lu) in the stroma (S) and between the epithelial cells (E) Uncontrasted section ×6300 B and C Electron micrographs from small vessels of the ciliary body base Peroxidase reaction product is present in the vessels (Lu) but not in the stroma (S) The endothelium (En) is not fenestrated B Uncontrasted section ×5560 C Uranyl and lead ×9160

of the endothelial cells were not detected and there was no sign of passage of peroxidase through the intercellular clefts Accordingly a diffusion barrier to peroxidase is located to the endothelium of these vessels A few endothelial vesicles contained reaction product but none were seen to be opening to the stromal side within the time limit of this study

Discussion

The present study shows that horseradish peroxidase when injected intravenously is unevenly distributed in the anterior uvea Large amounts of peroxidase reaction product were demonstrated in the ciliary and iridial processes and in the choroid whereas only traces were found in the stroma of the iris and the ciliary body base

This distribution pattern must partly be explained by different protein permeability of the vessels in these tissues Thus the non fenestrated iris vessels are impermeable to horseradish peroxidase (Shiose 1971 Smith 1971 Vegge 1971 b) as are the retinal vessels (Shiose 1970) The fenestrated vessels of the ciliary processes and the choroid on the other hand are highly permeable to this tracer (Shiose 1970 Smith 1971 Vegge 1971 a) The present study has shown that the vessels of the ciliary body base are non fenestrated and impermeable to horseradish peroxidase like the vessels of iris and retina Apparently detailed studies of these vessels have not been reported before Taniguchi (1962) however found no pores in the endothelial cells of those capillaries of the ciliary processes that were deeper in location

The sharp demarcation of peroxidase reaction product between the processes and the stroma of the iris and the ciliary body base indicates that there is a functional barrier against diffusion of peroxidase from the processes across these tissues The present study however does not reveal the nature of this barrier This problem will be dealt with separately

The present findings appear to be consistent with observations on the distribution of immunoglobulins and albumin in the ciliary body and iris of the human eye as described by Allansmith et al (1973) They found a marked difference in concentration of the serum proteins in the ciliary processes and the rest of the ciliary body and iris and they suggested a possible washout of proteins through the iris as an explanation of this We would be inclined to explain the protein distribution pattern as being due to a low protein permeability of the vessels of the iris and the ciliary body base as well as a low rate of movement of the

proteins through these tissues The differences of peroxidase activity in the tissues also seem to be in accordance with Bill s findings (1968) that the extra vascular protein space is relatively much larger in the ciliary processes than in the rest of the anterior uvea

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FAMILIAL ASTEROID HYALITIS

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Three brothers are presented with bilateral asteroid hyalitis. The condition was mild in one vitreous and hence a weak penetrance is suggested. This is the fourth report of familial cases and thus an inherited aetiology in asteroid hyalitis is emphasized again. The same family presented also two cases with medullated nerve fibres.

Key words: family history – asteroid hyalitis – vitreous opacity – medullated nerve fibres.

In asteroid hyalitis, or Benson's disease, or scintillatio nivea, minute white or yellow solid bodies are seen in essentially normal vitreous. They move as the eye is rotated but return to their original positions after limited excursions. The condition was differentiated from synchysis scintillans by Benson (1894) and the topic has been recently summarized by Wichser (1968). The aetiology is unknown although several reports have connected the disease with hypercholesterolemia (Gallenga 1931, Rieger 1937, Corrado 1948, Emelli 1952, Smith 1958), diabetes (Smith 1958, 1965, Agarwal et al. 1964, Bard 1964), arteriosclerosis (Bloch 1969, Verhoeff 1921, Holloway 1922, Luxenberg 1969, Brand & Winkler 1974) and high serum calcium values (Jervey & Anderson 1965). Contrariwise no relationship to hypercholesterolemia or diabetes was found by others (Hat-

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field et al 1962 Jervey & Anderson 1965 Luxenberg & Sime 1969 Brand & Winkler 1973) Histochemical analyses of asteroid deposits have shown calcium palmitate or stearate to be at the centre of these deposits (Rodman et al 1961) Hereditary factors have usually been totally neglected (cf Duke Elder 1969) although three reports of familial cases have been documented (Bietti 1937 Belicard 1958 1960 Tittarelli 1960) and inheritance as a possible aetiology has been recently stressed by Wichser (1968)

In the following report three brothers with bilateral asteroid hyalitis are presented and hence an inborn error of metabolism as an aetiological factor is again suggested The same family also provided two cases with medullated nerve fibres

Material and Methods

The proband of the asteroid family presented himself in December 1973 and a subsequent family study revealed two additional cases Ophthalmological study was performed under mydriasis and with a Haag Streit 900 slit lamp Special attention was paid to the possible presence of corneal dystrophies pseudo ex foliation syndrome and retinitis albipunctatus but none of these however was observed Some laboratory tests were performed (see below)

Results

Family S (Fig 1) The family came from Hyvinkaa town South Finland and consisted of seven sibs of whom five were examined

Case 1 The proband 67-year old man V o a = +1 75 = 1 0 Pronounced asteroid hyalitis was noticed in his right eye but only few asteroid bodies were seen in the left eye In the left eye a white patch of medullated nerve fibres with a feathered edge extended nasally from the margin of the disc The length of this patch corresponded to approx the diameter of the disc Tensions 17 mmHg (Schiotz) Serum cholesterol was 405 mg/ 100 ml before treatment and during the present clofibrate therapy at the 240 mg level He had intermittent claudication and he could walk approx 100 meters without pain. Bad memory Weight 82 kg height 167 cm gargoyle like habitus with large head and short neck

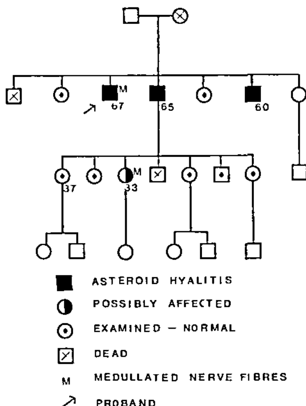


Fig 1
Family S Three brothers are affected with bilateral asteroid hyalitis

Case 2 65 year old man the brother of case 1 V o dx = +3 0 = 1 0 V o sin = +3.5 = 1 0 Right eye only five asteroid bodies Left eye pronounced asteroid hyalitis of white colour Tensions 17 mmHg Serum cholesterol 244 mg/100 ml triglycerides 156 mg/100 ml phospholipids 303 mg/100 ml Weight 9 kg height 165 cm adipose habitus Decreased hearing

Case 3 60 year old man the brother of the former V o dx = −0 25 = cyl + 0.5 ax15° = 1 0 V o sin = +0 25 = cyl + 0.5 ax170° = 1 0 Right eye only two asteroid bodies in the anterior parts of the vitreous Left eye hundreds of white round or roundish asteroid bodies attached to vitreous fibrils Chronic blepharitis Pronounced arcus senilis Drusen and pigmented disseminated areas of macular degenerations in both eyes covering an area of approx 3 disc diameters Serum cholesterol [illegible]8 mg/100 ml triglycerides 97 mg/100 ml phospholipids [illegible] mg/100 ml [illegible] hrs glucose tolerance test fasting 95 mg • 1 hr 146 mg 2 hr 166 mg Weight 65 kg height 16 cm normal habitus

The 71 and 63 year old sisters had no asteroid hyalitis but the former had cholesterol 470 mg/100 ml arterial hypertension and bilateral senile cataract and the latter had a large nonpigmented macular degeneration in her right eye and she had had a cardiac stroke one year earlier Accordingly several sibs showed signs of arteriosclerosis or hypercholesterolemia but these do not run entirely parallel with the asteroid hyalitis

Case 4 33 year old woman The third daughter of case 2 V o dx = -0 75 = 1 0 V o sin. = -1 0 = 1 0 Both ocular fundi presented patches of medullated nerve fibres from the margins of the disc with a length of approx. half disc diameter Both her lenses exhibited small grey opacities segmentally in the lower periphery as in congenital lamellar cataract. Both her vitreous were filled with innumerable minute round opacities attached to vitreous fibrils and seen only in the slit lamp but invisible by indirect ophthalmoscopy Hence the condition may present a pre stage of asteroid hyalitis but this diagnosis could not be settled with any degree of certainty

The other children of case 2 (see Fig 1) had no ocular abnormalities Their ages ranged from 37 to 21 years

Discussion

The prevalence of asteroid hyalitis has varied from 0 042 % (Görduren 1948) to 0 5 % (Hatfield et al 1962) in normal" population but was 1 91 % (eight cases out of 418) in the pseudo exfoliation material of Tarkkanen (1962) The rarity of the condition makes it highly improbable that there are several affected cases in the same family purely by chance – as was also emphasized by Tittarelli (1960) The bilaterality of the condition also strongly favours a dystrophic nature of the disease The penetrance at this age between 60 and 67 years seemed to be rather weak because the other eye in all three brothers was only mildly affected The mode of inheritance could not be decided and could be either autosomal dominant, recessive or X linked Noteworthy is that also in *the three earlier reports affected members were sibs* but *no cases were demonstrated* in two successive generations The reason may be the very late manifestation of the disease and hence the difficulty of finding two consecutive generations alive

The existence of two cases with medullated nerve fibres in the same family was interesting and has not been reported earlier in connection with familial cases of asteroid hyalitis There is a possibility that both have a common origin in a third factor In medullated nerve fibres also a hereditary tendency has been noted but is by no means commonly evident (cf Duke Elder 1964) Familial cases have been observed suggesting dominance (Meyer Riemsloh 1925 Kiso 1928 Cockayne 1936) or recessiveness (Catsch 1939 Waardenburg 1954) The

co existence of asteroid hyalitis and medullated nerve fibres may be coinciden tal but the rarity of both conditions (medullated nerve fibres 0 3–0 6 % Duke Elder 1964) does not favour any coincidence but merely some causational rela tionship such as a common metabolic genetic defect

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CATARACT INCISION

Knife incision versus stepped incision

BY

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A comparison was made of the postoperative results after cataract extraction with knife incision *ab interno* and stepped incision *ab externo* The complications related to wound healing were fewer when stepped incision was used There was no statistically significant difference between the groups in the degree of postoperative astigmatism Stepped incision *ab externo* seems to be preferable to knife incision *ab interno* in cataract surgery

Key words lens – cataract – ocular surgery

Several methods of incision technique are in use in cataract surgery (Barraquer et al 1964) At our clinic it has been made *ab interno* with Graefe s cataract knife under a limbus based conjunctival flap Unintentional filtration has been observed frequently at follow up examination As a lower incidence of unintentional filtration has been reported in conjunction with stepped incision *ab externo* than with knife incision *ab interno* (Witmer & Kreienbuhl 1971) the author changed his technique to stepped incision instead of knife incision The operative method was otherwise unchanged The object of this report was to compare the results obtained by these two incision techniques

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Methods and Material

The material consists of the results of 100 successive cataract operations in which the incision was made with a cataract knife *ab interno* and of 100 successive cataract operations performed subsequently using stepped incision *ab externo* Only the eyes in which elevated intraocular pressure required diathermy to provoke filtration and the eyes subjected earlier to filtrating glaucoma surgery were omitted

A binocular loupe with twofold magnification was used in the operations

Incision with Graefe s knife ab interno The pupil was dilated preoperatively with 2 % homatropine and 10 % metaoksedrine A solution of 1 % adrenaline was instilled into the eye several times immediately before the operation in order to prevent bleeding A limbus based conjunctival flap was prepared Possible bleeding sites were cauterised with a heated glass rod Using Graefe s knife an approx. 120° sclerocorneal incision was made The incision was continued in both directions with curved sclerocorneal scissors to make it a 180° incision A black 8–0 silk suture (SSC) was placed at the 12 o clock position in the wound and on both sides of it an 8–0 blue virgin silk suture (SSC) (Pohjanpelto 1974) The threads were drawn out of the wound and were looped aside Two peripheral iridotomies at 11 and 1 o clock positions were made Alfa chymotrypsin was injected under the iris and the wound and the anterior chamber were rinsed immediately afterwards with physiological saline solution The lens was extracted with a cryo extractor and the sutures placed in advance were drawn tight To produce miosis 0 01 % carbachol was injected into the anterior chamber with a curved 18 gauge lacrimal cannula (Beasley et al 1968 Pohjanpelto 1969) The sutures were tied and one 8–0 silk suture was placed on each side Five sutures in all were applied to close the wound Finally a small air bubble was injected into the anterior chamber and the conjunctival wound was closed with three 8–0 silk sutures

Stepped incision ab externo The operation was performed in the same way but the incision was made as follows A scleral groove about 0 4 mm deep was made with a razor blade immediately behind the surgical limbus The razor blade was placed at the tip of the razor blade holder (Moria) so that only 0 4 mm of the cutting edge was exposed The tip of the razor blade holder was then pressed against the sclera and the incision was made first from 9 to 12 o clock and then from 3 to 12 o clock A lamellar dissection about 2 mm wide was made towards the cornea with a Schaber scarifier (Fig 1) The anterior chamber was entered with the razor blade and the incision was completed with curved sclerocorneal scissors to 180° After this the first three sutures were placed and the operation continued as previously described

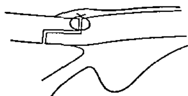


Fig 1
Stepped incision *ab externo*

The patient with both eyes covered was usually kept in bed until the day following the operation when one eye was uncovered and the patient was allowed to get up If the binoculus and keeping still caused the patient difficulties one eye was uncovered and the patient was allowed to get up earlier On the day following the operation local therapy was commenced with a combination of hydrocortisone and chloramphenicol (Scheroson f oftalmicum Leiras) three times daily These patients were discharged from hospital on the 10th postoperative day

The results of the follow up examinations 10–11 weeks after the operation are presented here in addition to the postoperative complications during the hospital stay The interval between the operation and the follow up examination was longer in a few exceptional cases

Refraction was determined by a Copeland streak retinoscope Astigmatism was generally examined also with a Javal keratometer The wound region was examined with a Haag Streit corneal microscope

Results

A technical drawback of the stepped incision compared with the knife incision was the longer duration of the operative procedure However after some experience the difference in time was reduced with the technique employed and was of no practical significance

The operating space within the anterior chamber was more limited with the stepped incision procedure as the site of entry to the chamber was more central This made it difficult sometimes to make the iridotomy openings peripheral

A complication with the stepped incision in one case was the rupture of the lens capsule when the anterior chamber was entered There were three other extracapsular operations in the stepped incision group but the incision method hardly affected what happened in these cases unless the narrowness of the operating space is considered to have contributed to it All the operations in the knife incision group were intracapsular

The main advantage of stepped incision was easier closure of the wound Placing the suture at a suitable depth after knife incision caused difficulties not encountered in stepped incision The step permitted precise placement of the sutures although no operating microscope was used

Table I shows the frequencies of complications that may be associated with wound healing They were lower with stepped incision than with knife incision On the whole the complications were not serious in either group

Hemorrhage in the anterior chamber disappeared spontaneously without surgical intervention in all the cases The only disadvantage observed was a

Table I
Postoperative complications related to wound healing after cataract extraction

	Anterior chamber hemorrhage	Shallow anterior chamber	Unintentional filtration
Knife incision (100 eyes)	14	10	18
Stepped incision (100 eyes)	7	6	8

prolongation of the hospital stay in some cases The inclusion of eyes in which blood remained in the anterior chamber intraoperatively contributed to the high incidence of hemorrhage

Shallowness of the anterior chamber was seen fairly frequently especially after knife incision The chamber was usually shallow rather than completely flat and the condition subsided quickly The treatment consisted of acetazolamide and mydriatics A drawback was the prolongation of hospital stay in some cases

As anticipated there were plenty of unintentional filtrations after knife incision The filtration area was generally small and shallow and caused the patient no subjective symptoms The filtration bleb was found to have disappeared in some of the eyes at a later follow up examination However a high cystic bleb developed in some cases causing the sensation of a foreign body in the eye Resuturing of the wound was therefore performed on one patient 14 months after the cataract extraction Unintentional filtration was less frequent after stepped incision and the bleb was generally small In some cases it could

Table II
Postoperative astigmatism after cataract extraction

Astigmatism (diopters)	0–1.0	1.25–2.0	2.25–3.0	3.25–	Mean astigmatism
Knife incision (no of eyes)	54	26	15	5	1.31 ± 1.04
Stepped incision (no of eyes)	47	27	13	13	1.58 ± 1.33

be demonstrated with certainty only with a slit lamp Therapeutic measures were not regarded as indicated in a single case

No patient of either group had prolapse of the iris One patient in the knife incision group underwent exploration of the wound on the 5th postoperative day and additional sutures were placed because of peaked pupil and subconjunctival infiltration but no prolapse of the iris was seen in the wound

There was no statistically significant difference ($P > 0.1$) between the groups in the degree of postoperative astigmatism Hence it may have been a coincidence that severe astigmatism over 3 0 diopters was a more frequent finding after stepped than after knife incision The results were otherwise similar for both groups (Table II) Astigmatism following knife incision was against the rule in 69 eyes with the rule in nine eyes and the remaining 22 eyes had no astigmatism The corresponding figures after stepped incision were 67 16 and 17

Discussion

Except for the incision technique all the operations were performed in the same way as far as possible and by the same surgeon Hence comparison of the results probably discloses the effect of the incision technique

In the study reported by Witmer & Kreienbuhl in 1971 astigmatism diminished when stepped incision was adopted in lieu of knife incision This was not found in the present series in which severe astigmatism was even more frequent after stepped incision It is possible that this was a coincidence but it motivates study of the situation in a more comprehensive material No routine determination of refractive power was made immediately after the operation but in some cases in which it was done high degree astigmatism against the rule was found to have developed later in the course of the postoperative 10–11 weeks Thus the astigmatism was probably not the result of incorrectly performed suturing but developed during the healing of the wound

The incidence of complications associated with wound healing were lower with stepped incision Unintentional filtration in particular decreased However unintentional filtration seemed to cause the patients only slight inconvenience and good results of its treatment have been reported (Gehring & Ciccarelli 1972 Yannuzzi & Theodore 1973) Filtration which occurs 10–11 weeks postoperatively may also disappear spontaneously The high incidence of unintentional filtration may have been caused by the topical cortisone treatment given postoperatively (Kirk 1974)

Although the other complications too associated with wound healing caused no major harmful effects in the present material the prognosis can be poor Stepped incision *ab externo* seems therefore to be preferable to knife incision *ab interno* The initial groove of the incision should perhaps be made more sclerally than was done in this series at least 1 mm behind the surgical limbus and not right beside it The lamellar part might also be narrower than the 2 mm used This would give a more peripheral entry into the anterior chamber and better operating space Corneal astigmatism would perhaps also decrease if the incision was made more sclerally (Jaffe 1973)

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ANTERIOR CHAMBER DEPTH AND PRIMARY ANGLE-CLOSURE GLAUCOMA

I An epidemiologic study in Greenland Eskimos

BY

P H ALSBIRK

The anterior chamber depth (ACD) was measured by optical pachymetry in 60 Eskimos with primary angle closure glaucoma (a c g) They lived in seven medical districts from nearly all parts of Greenland in locations with a total of 2 800 Eskimo inhabitants over 40 years Out of these an ACD survey recently described covered 1 072 The a c g patients had been ascertained by symptoms or through screenings
Prevalence rates are given according to epidemiologic method age and sex totalling 1 6 % in males and 5 1 % in females on an average 3 5 %
The risk of a c g at various levels of ACD was estimated in the survey population
The ACD mean value of the a c g patients closely agreed with that of the Caucasian series On the other hand in Eskimos the mean value of the general population was only 0 5 mm above the mean ACD found in a c g patients whereas in Caucasians this difference is reported to be about 1 0 mm An attempt is made to explain the ethnic variation and the sex difference with respect to prevalence of a c g simply in terms of the ACD distribution

Key words glaucoma – angle closure glaucoma prevalence – sex age screening methods – anterior chamber depth – Greenland Eskimos

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The global epidemiology of primary angle closure glaucoma (a c g) is known to a limited extent only Recent biometric research in Caucasians has improved our knowledge of the anatomical traits predisposing to angle closure The anterior chamber depth (ACD) is still the most deviating quantitative parameter in such studies closely associated with narrow occludable chamber angles and risk of pupil block (Rosengren 1931 Delmarcelle et al 1969 Lowe 1970 Storey & Phillips 1971) However due to the relatively low prevalence of a c g in Caucasians there has been no epidemiologic study so far which has described the a c g occurrence on the background of the ACD distribution within the population examined Even the well known female preponderance in all samples of a c g patients has not as yet been satisfactorily accounted for in terms of biometric sex difference (Lowe 1972)

Recently unequalled prevalence rates have been reported in Eskimos from Canada and Greenland (Drance 1973 Clemmesen & Alsbirk 1969 1971 Clemmesen 1973 Alsbirk 1973) A c.g has hereby been depicted as a major health problem and a challenge to the health service in the arctic areas which are mostly remote from ophthalmic care by specialists

This situation motivated the *ACD survey* in Greenland Eskimos described recently A rather uniform low level of ACD in various districts and a conspicuous variation with age and sex was found (Alsbirk 1974 b a)

In this paper the a c g prevalence in terms of ACD distribution is described also with a view to the influence of sex age and methods of detection

Material

The a c g patients of the study had been found because of *symptoms* (37) or through population *screening* (23) and this subdivision was maintained throughout In Table I the composition of the material and the population basis are given The total group found due to *symptoms* is shown in *part A* These 37 patients lived in the preselected towns and villages listed below the Table and had previously been diagnosed in the seven medical districts represented All patients already known and still alive at the time of the study were examined

Details concerning the *ACD survey subsamples* are given in *part B* A total of 1 072 Eskimos over the age of 40 had been examined with a participation rate of 93 % in the groups selected (Alsbirk 1974 b) Among the 1 072 persons

Table I

Population basis number of patients with primary angle closure glaucoma (a c g) and prevalence rates according to methods of detection

Material	♂ no	♂ %	♀ no	♀ %	Total
*A					
Census population of a c g survey aged 40+	*1994*		*1461*		[illegible]
A c g patients known previously due to *symptoms*	1	[illegible]	30	*2.1 %*	3
B					
Subsamples selected for *ACD survey* aged 40+					
1) *Umanaq district* population examined	*215*		*213*		*428*
A c g patients known previously due to *symptoms*	1	*0.5 %*	10	*4.7 %*	11
A c g patients found in *screenings*					
a) tonometry gonioscopy	1	*0.9 %*	10	*5.? %*	11
b) ACD survey	1		1		2
Total a c g patients	3	*1.4 %*	21	*9.9 %*	24
⁹) *Other locations* population examined	*89*		*555*		*644*
A c g patients known previously due to *symptoms*	1	*1.1 %*	9	*1.6 %*	10
A c g patients found in *screening* (ACD survey)	1	*1.1 %*	9	*1.6 %*	10
Total a c g patients	2	*2.2 %*	18	*3.2 %*	20
1 + 2) ACD survey population examined	*304*		*68*		*10 2*
Total a c g patients	5	*1.6 %*	39	*5.1 %*	44

A Census population located in Angmagssalik district Sydprøven Julianehåb Sukkertoppen Egedesminde Jakobshavn Umanaq district Upernavik and Augpilaqtoq

B ⁹) Population in Angmagssalik Kap Dan + Kungmiut (89 ♂ and 94 ♀) and Julianehåb Sukkertoppen Egedesminde Upernavik + Augpilaqtoq (461 ♀ only) cf Alsbirk (1974 b)

Table II

Age at detection and duration of disease at ACD measurement for 60 a c g patients

A c g detected by	No of patients	Age at diagnosis median	Age at diagnosis range	Duration of a c g (years) median	Duration of a c g (years) range
Symptoms acute attacks	17	54	36–69	5	1/2–18
Symptoms other	20	56 1/2	44–72	3	1–12
Symptoms all	37	54	36–72	4	1/2–18
Screening (tonometry gonioscopy)	11	58	39–75	2	0–2
Screening (ACD survey)	12	69	44–83	–	–

altogether 44 patients were detected of whom 21 (11 + 10) had been found earlier due to *symptoms* (and thus were already included in part A) The other 23 a c g patients ((11 + 2) + 10) were ascertained through *screenings only* as described below (see Methods)

Thus a total of 60 a c g patients emerged 44 belonging to the ACD survey groups (21 by symptoms 23 through screenings) and 16 from the remaining population (by symptoms)

In Table II the *symptom and screening groups* are shown according to *age at diagnoses* and *duration* of disease up to the ACD measurement

Table III shows the composition of the material according to the *diagnostic stage* of single eyes Out of the 60 patients 32 had bilateral and 28 unilateral a c g In 44 patients the ACD of both eyes was measured the average values being used while in 16 patients only one eye was included The fellow eyes could not be measured due to aquired deformity (12 eyes) enucleation (3) and aphakia (1) in 12/16 of the cases following acute attacks Thus ACD values of 104 eyes were available for the study i e 78 eyes with a c g and 26 fellow eyes so far without a c g

Surgical treatment had been applied to 42 (40 %) of the eyes measured peripheral iridectomy in 26 and more extensive or filtrating operations in 16 eyes Seven of the interventions had been performed prophylactically in fellow eyes so far without a c g *Medical treatment* – nearly always pilocarpine 2 % – was used in six of the operated eyes and in 11 of the 62 unoperated eyes Of these eyes 24 were measured and a c g diagnosed subsequently at the ACD survey As to the rest (27/62) 19 eyes and probably another eight eyes did not for various reasons receive medical treatment at the time of ACD measurement

Table III

Diagnostic classification of 92 eyes (60 patients) according to the stage of a c g at detection

A c g detected by	No of eyes and stage of a c g					Total	
	Latent	Intermittent	Acute	Chronic	Unspecified	Eyes	Patients
Symptoms	2	20	23	11	1	57	3
Screening (tonometry and gonioscopy in Umanaq)	1	6	–	10	–	17	11
Screening (ACD survey in sex districts)	1	15	–	2	–	18	12
Total eyes with a c g	4	41	23	23	1	92	–
Total patients with a c g	4	24	17	15	–	–	60

The *visual acuity* was not examined at the time of the study but the medical files showed that 16 % of the eyes (19/120) and 8 % of the patients (5/60) were blind (visual acuity $\leq$ 4/60) In both eyes of one patient and one eye of 10 patients no perception of light had been found

Methods

The ACD measurements in this study were performed by optical pachymetry using Haag Streit instruments as described in detail elsewhere (Alsbirk 1974 b c)

The diagnostic classification in Table III was based on the criteria given by Clemmesen (1971) *The diagnostic stages were defined in the following way*

Latent a c g Patients with occludable chamber angles without subjective symptoms but with a positive gonioscopic and tonometric response to provocative tests

Intermittent a c g Patients with occludable angles and intermittent observed pressure rises accompanied by prodromal symptoms such as headache haloes and blurred vision but with normal tension in the interparoxysmal periods

Acute a c g Patients with severe attacks of congestive ocular hypertension confirmed as depending on occludable chamber angles

Chronic a c g Patients with at least partially occluded chamber angles and with permanently increased intraocular pressure (IOP)

The *symptom* cases had primarily been ascertained by the medical officers and/or the annually visiting ophthalmologists The organization of ophthalmic care in Greenland has been described by Clemmesen (1973)

Two different *screenings* resulted in the diagnosis in the rest of the patients 1) *Tonometry* and – in a subsample – *gonioscopy* were carried out in the Umanaq population (Table I B 1) Triple test (Kirsch 1965 Kristensen 1967) or other provocative tests were used in eyes with IOP ≥ 24 mm (Schiøtz scale values ≤ 5 1/2/7 5) and in normotensives *with narrow chamber angles* (Clemmesen & Alsbirk 1969 1971 Clemmesen 1971 Alsbirk 1970) 2) *ACD measurement* followed by a *darkroom test (DRT)* was used as an a c g screening procedure during the ACD survey In Umanaq all persons with an ACD value below the lower 95 % limits given by Tornquist (1953) were submitted to DRT when gonioscopy and – if necessary – provocative tests had not been made before In the other districts (Table I B 2) a simpler somewhat restricted selection procedure for DRT was used an ACD value below 2 0 mm As no ophthalmic surgeon joined the ACD survey more effective provocative tests could not be applied Therefore the DRT was immediately repeated for another hour when only borderline increases of about 8 mm were found In the 12 new cases thus detected an ophthalmologist (Clemmesen) confirmed the gonioscopical observations and diagnoses a few months later

Statistical methods are described elsewhere (Alsbirk 1974 b) In order to permit pooling of individual ACD results from various sex age and location groups a standardized deviation score (DS) was computed for each person Thus the difference – positive or negative – between the individual ACD value and the appropriate regression line of ACD on age was calculated with the standard deviation ($s_{y\,x}$) as unit of measurement

Results

Prevalence rates of primary angle closure glaucoma according to sex location and age

The prevalence rates at the time of the ACD survey are included in Table I As to the influence of *sex* two assessments were obtained The females significantly outnumbered the males in the symptom group (A) ($\chi^2 = 10\,7$ $P < 0\,01$) *with a ♀/♂ sex ratio of 3 8* In addition both sexes were uniformly screened in two districts – Umanaq and Angmagssalik – whereby 14 a c g cases 3/304 ♂ and 11/307 ♀ were identified However this difference was not significant at the 0 05 level (Fisher s exact test $P = 0\,057$ two tailed)

The *geographical variation* is not given in detail as the figures were small even for females However in the symptom group the prevalence of a c g was 4 7 % in Umanaq females – a finding which motivated the whole study of glaucoma in Greenland – against 1 6 % in the six other areas ($\chi^2 = 6\,1$ $P < 0\,025$) As to the cases found through screenings a similar comparison could not be

made due to the different methods used in Umanaq and outside but taken at face value the corresponding rates differed considerably (5.2 % and 1.6 %). The final female prevalence rate in the areas examined in the same way (Table I B 2) was also remarkably high 3.2 % (95 % confidence limits 2 %–5 %).

Tables II and IV show the considerable influence of *age*. Two patients had been diagnosed before the age of 40 and half of the symptom group before the age of 54. The prevalence rates showed a pronounced increase with age. Above the age of 60 every twelfth woman 8.3 % of the combined ACD survey population suffered from a.c.g.

Prevalence rates of primary angle closure glaucoma according to level of ACD

In the subsamples selected for the ACD survey (Table I B) a total of 44 a.c.g. patients was found. In Table V and Fig. 1 the ACD values of these patients are given as a part of the total male and female distributions obtained in the population survey. The patients all belonged to the lower half of the ACD distributions. The male patients – although few in number – constituted nearly the same high proportion of the population classes as the females in the lower

Table IV

Female prevalence rates as in Table I specified according to age given as proportions and rounded percentual values

A.c.g. patients detected by	Age groups				
	40–49	50–59	60–69	70+	Total
Symptoms (A)	$\frac{3}{638}$ 0.5 %	$\frac{9}{362}$ 2 %	$\frac{14}{310}$ 5 %	$\frac{4}{151}$ 3 %	$\frac{30}{1461}$ 2 %
All methods Umanaq (B 1)	$\frac{3}{80}$ 4 %	$\frac{7}{56}$ 13 %	$\frac{9}{58}$ 15 %	$\frac{2}{19}$ 11 %	$\frac{21}{213}$ 10 %
All methods other locations (B 2)	$\frac{1}{157}$ 1 %	$\frac{4}{186}$ 2 %	$\frac{6}{140}$ 4 %	$\frac{7}{79}$ 10 %	$\frac{18}{555}$ 3 %

Table V

Distribution of ACD in 44 a c g patients and in their background population (1067 Eskimos aged 40+) ACD specified prevalence rates are shown in per cent

Lower class limits of ACD (mm)	♂ A c g patients No	♂ A c g patients Per cent of class	♂ Population No	♀ A c g patients No	♀ A c g patients Per cent of class	♀ Population No
3 3						1
3 2						1
3 1			3			2
3 0			9			5
2 9			7			13
2 8			16			1[illegible]
2 7			31			34
2 6			23			62
2 5			3[illegible]			76
2 4			37			85
2 3			44	1	*1 %*	101
2 2			26	1		83
2 1			29		*3 %*	79
2 0			14	4		70
1 9	1	*11 %*	14	6	*14 %*	47
1 8	1		4	6		39
1 7	1	*33 %*	5	6	*40 %*	[illegible]1
1 6	2		4	8		14
1 5			1	2	*33 %*	9
1 4				2		3
1 3				2	*100 %*	2
1 2						
1 1				1		1
Total no n	5	*1 6 %*	302	39	*5 1 %*	[illegible]
Mean age $\bar{x}$	68		53 [illegible]	61		55 9
Mean ACD y	1 76		2 43	1 [illegible]6		2 29
S D s_y	0 13		0 31	0 23		0 32
Stand error $s_{\bar{y}}$	0 06		0 02	0 04		0 01

tail of the distributions Use of an arbitrary 2 0 mm limit thus revealed that about 18 % of males (5/28) and 24 % of females (33/136) below that level were *a c g patients The proportion of* a c g patients at a given ACD level is shown in Table V to be steeply increasing with decreasing ACD

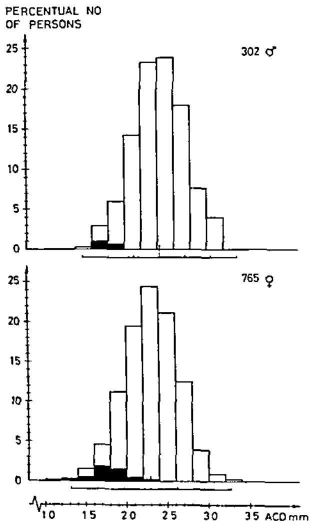


Fig 1

Percentual ACD distribution in 1 067 Greenland Eskimos over 40 years For each sex the mean value (↑) ± *3 standard deviations are shown Shaded part of the histograms* indicates the proportion of a c g patients within the sample

The ACD distributions of Table V are given simply as they were found in each sex without correction for age The mean age of the a c g patients was on an average 15 (♂) and 5(♀) years above the mean age of the population examined However this age difference could only explain s small part of the mean deviation observed (0 07 mm in males and 0 05 mm in females according to linear regressions on age of the patients)

In Table VI each patient of the total material is represented by the standardized deviation score (DS) After this transformation the population distribution showed a Gaussian distribution with mean at zero and standard deviation at unity The mean value of the a c g patients was –1 5 DS units i e significantly lower than the population mean Their standard deviation was significantly smaller than the population value (F = 1 93 $P < 0\ 01$) The different methods gave subgroups with rather similar results As 1 DS unit corresponds to 0 31 mm ACD the average deviation of the 60 patients was –0 45 ± 0 03 mm (mean ± standard error) with standard deviation 0 22 mm

Discussion

The chief aspect of this epidemiologic study was the relationship between the prevalence of a c g and the ACD distribution of a given population Greenland Eskimos constituted a most favourable study group due to their cooperability high prevalence of a c g and well known ACD distribution

Table VI
Standardized deviation score (DS) of ACD in the population and in the sample of 60 a c g patients according to method of detection

DS distribution		Population values	A c g patients detected by				
			Symptoms acute	Symptoms other	Screening tonometry gonioscopy	Screening ACD+DRT	Total
No	N	1067	17	20	11	12	60
Mean	x	0 00	–1 6	–1 5	–1 5	–1 3	–1 48
S D	s_x	1 00	0 8	0 8	0 6	0 5	0 72
Stand error	$s_{\bar{x}}$	0 03	0 2	0 2	0 2	0 1	0 09

As to the a c g prevalence the rates obtained were influenced by *age* and *sex* Furthermore the *methods of detection* influenced the estimates as Tables I and IV show Previous cases diagnosed because of acute attacks and other *symptoms* clearly represented only the top of the iceberg New cases were revealed by the *screenings* to a varying degree Obviously the tonometry gonioscopy provocative test procedure in Umanaq augmented the number of cases remarkably A relatively smaller yield was obtained in the five areas where only a darkroom test was applied in persons with ACD below the 2 0 mm level However the cases ascertained by this procedure showed impressive IOP rises ranging from 22–48 mm (in five patients due to secondary rises to 39–64 mm IOP by the repeated test) It is a well known fact that false negatives frequently occur even with the more effective provocative tests (e g Lowe 1967) Therefore the prevalence rates at least outside Umanaq must be minimum values

It can be calculated that the overall prevalence rates of the ACD survey population – 1 6 % in males and 5 1 % in females – would imply a total of 250 a c g patients in Greenland in 1972 i e 3 5 % of the 7 250 Eskimos above the age of 40 Thus judged by the statistics given by Clemmesen (1973) probably about 100 cases had not yet been diagnosed

In *Canadian Eskimos* similar prevalence rates were recently found by Drance (1973) In 377 persons over 40 years he found 11 patients with primary a c g (2 9 %) using tonometry and gonioscopy but no provocative tests A comparison between the ACD level in Greenland and Canadian Eskimos showed very similar low levels (Alsbirk & Forsius 1973 Alsbirk 1974 b)

In *other ethnic groups* prevalence rates of a c g are not well known With the overwhelming majority of glaucoma screenings only open angle glaucoma cases have been searched for An exception was the Welsh population study made by Hollows & Graham (1966) showing only four cases of a.c.g (0 09 %) among 4 600 inhabitants aged 40–75 If – tentatively – this estimate is considered to be representative for Europeans the Eskimo a c g prevalence is nearly 40 times greater as pointed out by Drance (1973) This remarkable ethnic difference applies also to Greenland Eskimos

As to the main topic of the study – the association between ACD and risk of a c g – the *reliability of the ACD value* also in diseased eyes was a critical point

A few investigations have dealt with this problem and neither the disease itself nor the surgical intervention seem to change the *axial* ACD significantly (Tornquist 1956 Auricchio 1959 Aizawa 1960 Grieten & Weekers 1962 Luglio 1973) Therefore the inclusion of operated eyes without deformity was justified and both eyes of the patients could be included even in unilateral cases as the best measure of the individual – A few eyes (17) were influenced by pilocarpine –% but were nevertheless included Rosengren (1931) found no reduction of ACD due to pilocarpine over the age of 45 but Wilkie et al (1969) demonstrated a slight reduction (0 1 mm) during pilocarpine

treatment of elderly people over 60 and a corresponding finding was recently made by Abramson et al (1973) After all the ACD values obtained in the a c g sample also could be taken as fairly reliable anatomical measures

In Table VII a series of biometric studies in a c g patients is shown Generally the ACD mean value in the Eskimo *patients* are very close to those of Caucasian and Japanese patients when the different methods are accounted for On the other hand the ACD mean value in the Eskimo *population* is much lower than those of other population groups (see Controls of Table VII) Thus a difference of only half a mm between population and patients was found in *Eskimos against 0 9–0 13 mm in the other ethnic groups*

What are the implications of an epidemiologic study like the present for other populations? As Lowe (1973) pointed out the examination of patients in general ophthalmic practice reveals that many people carry the *predisposition for* a c g *without* developing the disease but at present the risk of a c g on different ACD levels is not known Tornquist (1956) tried to estimate the relative risk of

Table VII

ACD parameters in various materials of a c g patients compared with controls

	A c g patients			Controls			Mean difference in	
	n	y	s_y	n	y	s_y	mm	s_y units
Swedes[a] (Tornquist 1956)	71	1 59	0 28	98	2 6	0 28	1 0	3 0
Italians (Auricchio 1959)	25e	1 82	0 34	50e	3 08	0 42	1 3	3 1
Japanese (Aizawa 1960)	84e	2 33[b]	0 32	99e	3 2[b]	0 29	0 9	3 1
Belgians (Delmarcelle et al 1969)	78e	1 90	0 29	151e	2 80	0 34	0 9	2 6
Australian Whites (Lowe 1970)	61	1 8	0 25	80	2 8	0 36	1 0	[illegible]
Eskimos ♀ (Alsbirk)	50	1 77	0 23	765	2 29	0 32	0 5	1 6

a *Acute glaucoma* b Corneal thickness included c Ultrasound data
n No of persons (or e eyes) y Mean ACD (mm) s_y Standard deviation (mm)

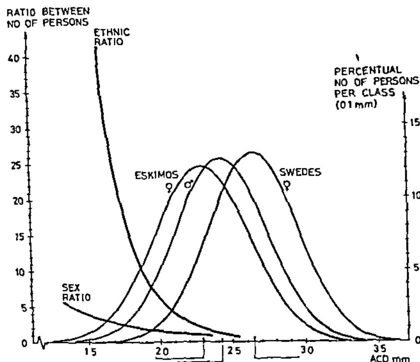


Fig 2

Gaussian distributions of ACD calculated in female and male Eskimos and female Swedes (based on Tornquist (1953) 98 females aged 50 and 65 years) Means and standard deviations are indicated *Ethnic ratio* shows the effect of a large ACD mean difference (0.36 mm) upon the ratio of the no of Eskimo to Swedish females at the lower levels of ACD *Sex ratio* shows the effect of the sex difference (0 13 mm) upon the ratio of the no of females to males

contracting acute glaucoma on different levels of ACD Thus the risk seemed to increase from a value of 1 (arbitrarily chosen for ACD 2 0–2 5 mm) to 175 (ACD 1 5–2 0) and nearly 100 000 (ACD 1 0–1 5) The present study for the first time gives the risk as the proportion of a c g patients at various levels of ACD obtained as an empirical result of a population study Thus the percentual *prevalence rates at various ACD levels* as given in Table V constitute an important result and these probabilities or *risk estimates* might become relevant in the care of persons with shallow chambers in any population In a few years a follow up study of the relation between the level and decrease of ACD and the incidence of new a c g cases would probably be of still greater value

The ratio of a c g prevalences in Eskimos and Caucasians was shown to be about 40 In nearly all clinical studies of a c g patients a corresponding but smaller sex ratio between the number of females and males has been found In 11 papers surveyed by Duke Elder (1969) the females made up 64–80 % of the series and the present study is in close agreement

This epidemiologic pattern seems to be highly associated with the level of the ACD distribution in Eskimo and Caucasian populations as it is in females and males The association is illustrated in Fig 2 The relative proportion of normal Swedish females inside the risk zone of ACD values is far smaller than the part of the Eskimo distribution found here Thus the *ethnic ratio* curve shows that there are about 40 times more Eskimo females around the general ACD mean value of a c g patients An analogous agreement with the prevalence pattern of a c g is illustrated by the *sex ratio* curve The relative excess of females clearly increases towards the lower ACD values showing about three times more females at the 1 7–1 8 mm level of ACD

Such a model – relating the a c g predisposition to the ACD distribution in a simple way – is admittedly fairly theoretical depending on assumptions which can not all be analysed Yet the curves of Fig 2 seem to be essential to an interpretation of sex difference and ethnic variation in a c g epidemiology

In conclusion the study showed that Eskimo a c g patients have shallow anterior chambers just like patients from other populations The very high prevalence in Eskimos seems to be simply inherent in the low level of their general ACD distribution Correspondingly the excess of females among a c g patients could be interpreted in terms of a sex difference in ACD The risk of primary angle closure glaucoma at various levels of ACD has been empirically estimated for the first time

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CEREBROSPINAL FLUID FINDINGS IN PATIENTS WITH OPTIC NEURITIS

BY

E NIKOSKELAINEN K IRJALA and T T SALMI

Cerebrospinal fluid specimens (CSF) from 59 patients with optic neuritis and from 59 age matched neurological control patients were studied The patients with optic neuritis showed significantly higher CSF IgG concentrations and a higher frequency of abnormal CSF electrophoresis as compared with the control specimens Elevated relative IgG values (expressed as percentage of CSF total protein concentration) were noticed in 42 %, abnormal electrophoresis in 41 %, pleocytosis in 26 % and increased total protein concentrations in 29 % of CSF specimens from patients with optic neuritis Thirty patients with an unknown cause of optic neuritis and without any neurological symptoms other than optic neuritis showed increased relative IgG in 40 %, abnormal electrophoresis in 31 %, pleocytosis in 33 % and increased total protein concentration in 27 % of CSF specimens A reduced serum/CSF albumin ratio was noticed in high frequency in patients with optic neuritis indicating possible blood brain barrier damage. There were however 13 patients with optic neuritis and two from the control group with normal serum/CSF albumin ratio but reduced serum/CSF IgG ratio This finding supports the presumption that part of CSF IgG is produced within the central nervous system (CNS)

Key words optic neuritis – multiple sclerosis – cerebrospinal fluid proteins – IgG – electrophoresis – CSF pleocytosis – CSF albumin – CSF complement – blood brain barrier damage

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Optic neuritis is a fairly common eye disorder the most common cause of which is multiple sclerosis (MS) Optic neuritis with its typical symptoms usually brings the patients to an ophthalmologist at an early stage The cause of optic neuritis however often remains unknown during the disease Later a considerable number of patients appear to suffer from MS

The diagnosis of MS up to now is a clinical one but the cerebrospinal fluid (CSF) of patients with MS shows some typical features supporting the diagnosis Forty per cent of all MS plaques are situated periventricularly i e near the CSF space (Tourtellotte 1970 a) The optic nerves are also surrounded by the sub arachnoideal space Immunoglobulin G (IgG) is probably produced locally within the central nervous system (CNS) in patients with MS The CSF may thus reflect the newly formed CNS protein by diffusion through the extracellular spaces The most valuable diagnostic methods at present are the quantitation of IgG and the protein electrophoresis of CSF As shown e g by Laterre et al (1970) the morphological pattern of gammaglobulins is more important than their absolute or relative values Along with these methods the pleocytosis and moderately increased total protein concentration in the CSF are fairly common abnormalities in MS

There are only a few reports concerning CSF abnormalities in patients with optic neuritis Link et al (1973) noticed pleocytosis in 44 % (18/41) increased total protein in 49 % (20/41) increased relative IgG concentration expressed as percentage of the total protein concentration in 34 % (14/41) and abnormal electrophoresis in 51 % (21/41) of CSF specimens from 41 patients with optic neuritis without any other neurological symptoms Sandberg & Bynke (1973) studied 25 CSF specimens from patients with isolated optic neuritis and noticed pleocytosis in 60 % an increased total protein concentration in 24 % elevated IgC value in 16 % and an abnormal gammaglobulin pattern in 24 % of the specimens

The present report describes the results of studies concerning pleocytosis total protein albumin IgG and beta 1A/beta 1C globulin concentrations (the third factor of the complement system) as well as the frequency of abnormalities in electrophoresis in CSF specimens from 59 patients with optic neuritis and 59 *neurological control patients*

Material

Patients with optic neuritis The clinical material consisted of 59 patients with optic neuritis all in the clinically active phase They were examined and followed up for at least 6 months by one of us (E N) at the Department of Ophthalmology University of Turku from 1970–1973 In 42 patients the disease was

unilateral 14 had bilateral optic neuritis simultaneously and in 3 patients the one eye was affected later but within one month s interval after the onset of optic neuritis in the other eye In 41 patients the disease was retrobulbar 13 had papillitis and in 5 patients the optic disc was blurred but without distinct oedema on admission thus presenting a borderline type between optic neuritis and papillitis In all eyes with optic neuritis there was an absolute or relative central or paracentral scotoma in the visual field studied with Goldmann s perimeter

Table I shows the patients with optic neuritis divided into five groups according to the possible cause of optic neuritis based on neurological consultation The patients in group I had according to the criteria of the Schumacher Committe (Schumacher et al 1965) been diagnosed as having possible or probable MS Group II included those patients who had suffered before or during optic neuritis from neurological symptoms suggesting the diagnosis of MS although the diagnosis had not been made before or during optic neuritis In group III there were two patients with chronic alcoholism and two patients with ischaemic papillitis Group IV included two patients with Guillain Barre syndrome one with viral meningitis complicated with bilateral optic neuritis and two patients

Table I
Patients with optic neuritis according to the possible cause

Patient groups	Number of patients		
	Males	Females	Total
I Patients with MS diagnosed before or during optic neuritis	1	4	5
II Patients with neurological symptoms suggesting the diagnosis of MS	6	9	15
III Patients with vascular or toxic cause	4	0	4
IV Patients with some other known cause of optic neuritis	4	1	5
V Patients with an unknown cause of optic neuritis	9	21	30
Total	24	35	59

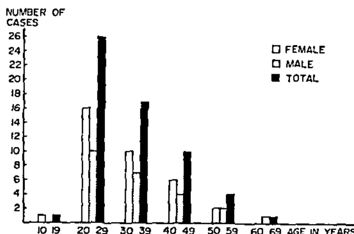


Fig 1
Age at onset of the disease and sex distribution in 59 patients with optic neuritis

with Leber s hereditary optic atrophy Group V included those patients in whom the cause of optic neuritis was unknown and who had no neurological symptoms or signs other than optic neuritis

Fig 1 shows the age and sex distribution of the material Most of the patients were young or middle aged

The control group This group consisted of 59 patients selected from patients examined and treated at the Department of Neurology University of Turku from 1970–1973 for some disorder other than MS or infectious diseases of the central nervous system (CNS) These patients were matched in age within 5 years and in sex with the patients with optic neuritis The diagnoses of the patients were as follows migraine (8) headache (5) epilepsy (9) psychoneurotic diseases (12) cerebrovascular diseases (11) mononeuropathy (1) dermatomyositis (1) amenorrhea (1) encephalopathy (2) facial neuralgia (1) Parkinsonism (2) vertigo (1) post traumatic syndrome (2) sciatica due to intervertebral disc disease (1) diplopia of unknown cause (1) cerebral atrophy (1)

The CSF and serum specimens The lumbar puncture was usually performed during the first week of admission to the hospital A blood specimen was also taken at the same time None of the CSF specimens contained visible blood The cell count immediately after lumbar puncture showed that in 11 specimens from patients with optic neuritis and in 8 from the controls the erythrocyte count exceeded 10 cells/mm^3 in patient group V the frequency was 6/30 respectively After cell counts and centrifugation the CSF supernatants were divided into

several smaller tubes in order to avoid any unnecessary freezing and thawing With the exception of the samples for protein electrophoresis which were stored at -20°C the serum and CSF specimens were stored at -40°C until analysis

The serum and CSF specimens from patients with optic neuritis and the matched controls were collected at about the same time so that the specimens from both patient groups were stored for a similar period before analysis

Methods

Cell counts were performed by standard procedures *The total protein* concentrations in the CSF specimens were determined with the modified biuret reaction (Hyvarinen et al 1972) *Albumin* and immunoglobulin G were estimated by single radial immunodiffusion (RID) according to Mancini et al (1965) Immunodiffusion was allowed to proceed at room temperature for 48 hrs in a moist chamber (Antiserum and standards were obtained from Behringwerke AG Marburg Lahn West Germany and agarose (Indubiose A37) from L Industrie Biologique Française France) The CSF *complement factor* beta 1A/beta 1C globulin was determined with electroimmunoassay (EID) (Laurell 1972) After RID or EID the agarose plate was dried washed and stained (Weeke 1973)

Before *protein electrophoresis* about 5 ml of CSF specimens were concentrated with the Amicon membrane ultrafiltration system (CF 50 A Amicon Corp Lexington Mass U S A) to a protein concentration of 5–7 g/100 ml The serum and CSF electrophoresis was carried out on gelatinized cellulose acetate membrane (Cellogel® Chemetron Milano Italy) using the Beckman Microzone electrophoresis cell apparatus A Barbital buffer (pH 8 6) time 50 min voltage 200 V and for protein staining the Coomassie brilliant Blue R 250 were used The electrophoresis was also carried out on agarose according to Vandvik & Skrede (1973) The amount of CSF in one patient with optic neuritis and three from the controls was not sufficient for electrophoresis

The significance of the differences between the mean values was tested with the two tailed Student s t test The Chi square test with the Yates correction was used to show distribution in frequencies

Results

Pleocytosis (Table II) The mean values of the leucocyte counts were not significantly higher in patients with optic neuritis than in the control group The frequency of leucocyte counts above 5 cells/mm³ was 15/58 (26 %) in patients

Table III

The mean values of relative CSF IgG concentrations and the ratios of serum IgG/CSF IgG and serum albumin/CSF albumin in patients with optic neuritis and in the control group

Patient groups		Relative CSF IgG expressed as a % of CSF total protein		Relative CSF IgG as % of CSF albumin		Serum IgG/CSF IgG		Serum albumin/CSF albumin	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Patients with optic neuritis									
Group I	(N = 5)	8.2	4.7	23.7	17.1	405	272	252	107
Group II	(N = 15)	13.4+++	5.2	26.8+++	9.2	260+++	142	210+++	122
Group III	(N = 4)	5.6	1.5	11.4	4.0	525(+++)	53	181	99
Group IV	(N = 5)*	6.9	2.1	18.7	7.6	249(+++)	68	144	69
Group V	(N = 30)	10.1++	6.9	28.8+	25.6	447+++	260	279+	180
Total	(N = 59)	10.2+++	6.1	25.8+++	19.9	386+++	232	243+++	125
The control group									
Total	(N = 59)	5.9	2.6	15.8	7.9	766	418	310	192

Symbols for statistical significance when compared with the control group
+ $P < 0.05$ ++ = $P < 0.01$ +++ = $P < 0.001$
(+) = the statistical significance uncertain because of smallness of the group
* in one patient the serum albumin and IgG values are missing

Table IV

Frequencies of increased CSF relative IgG concentrations and abnormal CSF electrophoresis in patients with optic neuritis and control patients

Patient groups	Relative CSF IgG concentration (expressed as a percentage of CSF total protein) above 10 %		Gelatinized cellulosa acetate electrophoresis			
			Increased gammaglobulin	Abnormal bands in gammaglobulin region	Abnormal electrophoresis with increased gammaglobulin and/or abnormal bands	
	No	%	No	No	No	%
Patients with optic neuritis						
Group I (N 5)	2	40(++)	3/5(+++)	3/5(+++)	3	60(++)
Group II (N 15)	10	67+++	10/15+++	8/15+++	11	73+++
Group III (N 4)	0	0	0/4	0/4	0	0
Group IV (N 5)	1	20	1/5	1/5	1	20
Group V (N 30)	12	40+++	8/29++	5/29	9	31+++
Total (N 59)	25	42++	22/58+++	17/58+++	24	41+++
The control patients (N 59)	3	5	2/56	2/56	4	7

Symbols for statistical significance when compared with the control group

++ $P < 0.01$ +++ = $P < 0.001$

(++) and (+++) the statistical significance uncertain because of smallness of the group

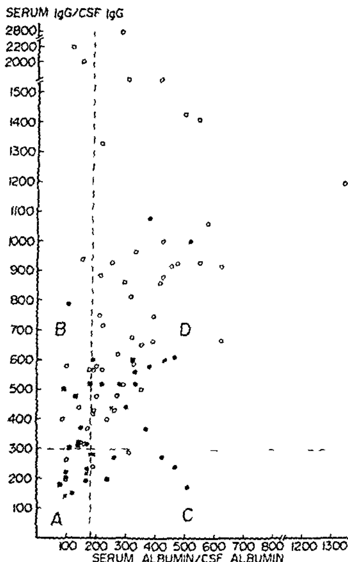


Fig 2

Electrophoretical results (Table IV Fig 3) Fig 3 shows a normal CSF electrophoresis from a control patient and an abnormal CSF electrophoresis from a patient with optic neuritis studied with gelatinized cellulose acetate membrane Table IV indicates abnormalities in the CSF electrophoresis in different patient groups Except for four CSF specimens which showed abnormal gammaglobulin bands in gelatinized cellulose acetate membrane but not in agarose electropho

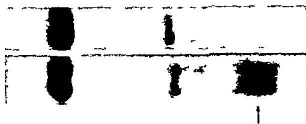


Fig 3

Above the Cellogel® electrophoresis from the CSF specimen of a control patient showing normal gammaglobulin pattern Below the Cellogel® electrophoresis from the CSF specimen of a patient with optic neuritis with abnormal bands (indicated with an arrow) in the gammaglobulin region

resis the result was the same with both supporting media. In three serum specimens from patients with optic neuritis and two from the control group there was an increased gammaglobulin content in serum electrophoresis with gelatinized cellulose acetate membrane All the other serum specimens showed normal electrophoresis

Discussion

According to the literature more than half of the patients with optic neuritis suffer from MS although the disease in many patients is benign (McAlpine et al

Fig 2

Relation between serum/CSF ratio for albumin and IgG in the patients with optic neuritis and the control patients Symbols Patients with MS or symptoms suggesting the diagnosis of MS (groups I and II together) crosses Patients with optic neuritis of other known causes (groups III and IV together) filled squares Patients with optic neuritis of unknown cause (group V) filled circles The control patients open circles

The broken lines show the lowest normal ratios of serum/CSF IgG and serum/CSF albumin The areas between the broken lines are marked with symbols A B C and D Patients in areas A and B represent cases with a low albumin ratio indicating possible blood brain barrier damage Patients in areas A and C represent patients with a low IgG ratio A low IgG ratio in patients in area A is possibly partly due to blood brain barrier damage only There were 13 patients (five from group V) with optic neuritis but only two from the controls who were placed in area A The patients in area C represent those with a selectively reduced IgG ratio They could represent cases in which CSF IgG is produced within CNS There were 13 patients (six from group V) with optic neuritis but only two from the controls in area C

however provide prognostic or diagnostic answers because the follow up period of the patients is still too short A re examination of the present clinical material after 5–10 years could possibly provide information about the correlation of CSF findings to the prognosis of optic neuritis

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THE DIRECTLY RECORDED STANDING POTENTIAL OF THE HUMAN EYE

BY

KLAS OLAV SKOOG

By means of a newly developed method including a suction contact lens and matched calomel half cell electrodes which were temperature stabilized the standing potential (SP) of the human eye considered to be generated mainly in the pigment epithelium could be directly recorded under stable conditions Upon a change in illumination from darkness to 16 Lux an initial rather fast negative transient was followed by slower damped oscillations with a frequency of about 2/hour The maximum amplitude of an oscillation was of the order of 5mV When the illumination was changed in the opposite direction the polarity of the oscillations was reversed into a mirror image of the variations described above Also the oscillations now were considerably smaller in amplitude With respect to phases and frequencies the results correspond well to the changes found in EOG measurements The new method seems to permit a study of the effects of drugs and other substances on the human SP which is also likely to reflect the condition of the pigment epithelium

Key words ocular electrophysiology – standing potential – clinical method – retina – pigment epithelium

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It has been known for more than a century (du Bois Reymond 1849) (frog) that there is a standing potential (SP) between the anterior and the posterior pole of the eye the cornea being positive to the fundus in vertebrates The present study describes a new method for the direct registration of the SP of the human eye without the aid of general anaesthesia

The human SP is generally measured indirectly with the electrooculogram (EOG) technique whereby electrodes at each canthus of an eye pick up potential variations produced when the eye being a dipole is moved in a regular way from side to side With such an arrangement Schott (1922) recorded eye movements but was not certain about the origin of the potentials Experiments performed by Mowrer Ruch & Miller (1936) showed that the electrical field was a projection of the SP Fenn & Hursh (1937) stated that the change in potential was linearly related to the sine of half the angle of eye movement According to North (1965) and others this formula is only an approximation which gives values above or below the theoretical values with large and small eye movements respectively The EOG technique was further developed and standardized by François Verriest & de Rouck (1955) Arden Barrada & Kelsey (1962) and Arden & Kelsey (1962)

An indirect measurement of the SP corresponding to the human EOG was performed in animals by Heck & Papst (1957) and by Horsten Philipszoon & Winkelman (1963) This method required passive movements of the eye of the animal and thus was a more complicated procedure than recording the human EOG in adults In contrast, direct SP recordings are much more difficult to obtain in man than in animals

Direct recordings of the SP from animals were carried out by du Bois Reymond (1849) (frog) Yoshida (1953) (toad) Muller Limmroth (1954) (frog) Kikawada (1968) (a variety of animals belonging to *Rodentia Aves Reptilia Amphibia* and *Pisces)* Knave Persson & Nilsson (1974 a b) (sheep) and others Yoshida (1953) and Muller Limmroth (1954) pointed out that lesions caused by the electrodes could influence the SP measurements

Because of eye movements electrode problems etc it has been very difficult to obtain stable direct SP registrations in man A first very crude recording was performed by Dewar & McKendrick (1876) who noted the existence of a potential The difficulties were summed up by Ermers & van Lith (1973) who stated that in humans the standing potential cannot be directly recorded This can only be done in an indirect way Working with the *c* wave of the human d c. registered ERG Knave Nilsson & Lunt (1973) Skoog & Nilsson (1974 a b) and Skoog (1974) obtained very stable d c recordings of slow potentials Finally the recording system was so stable that the author thought it worthwhile to register the SP with a modification of the d c ERG technique The *c* wave ampli

tude (Calissendorff Knave & Persson 1974 (sheep) Skoog & Nilsson 1974 b (man)) and the SP as reflected by the EOG potential (Kris 1958 Kolder 1959) both show oscillations with a frequency of about 2/hour They also react in the same way to certain drugs (Noell 1954 Heck & Papst 1957 Knave Persson & Nilsson 1974 a b and others) Since the *c* wave is built up mainly by the pigment epithelium (Noell 1954 Brown & Wiesel 1961 Steinberg Schmidt & Brown 1970 Schmidt & Steinberg 1971) which is also a major source of the SP (Noell 1954 Heck & Papst 1957 Gouras 1969) it should be interesting to correlate SP and *c* wave alterations in simultaneous registrations in man It is well known that the EOG potential is very sensitive to changes in the localization of the electrodes and skin resistance also interferes with the potential (Taumer Henning & Pernice 1974 and others) If the *c* wave and the SP were recorded from the same corneal and forehead electrodes changes that may arise at the electrode – body junctions would affect the recorded amplitudes of the *c* wave as well as of the SP simultaneously

A series of direct recordings of the human SP using a suction contact lens for the tip of the recording electrode matched temperature stabilized calomel electrodes and a low drift d c amplifier was carried out and the first results are presented in this paper Further standardization of the method is in progress

Material and Methods

The method for d c registration of the human ERG described by Knave Nilsson & Lunt (1973) and further developed by Skoog & Nilsson (1974 a b) and by Skoog (1974) was modified as described below in order to allow direct recordings of the human SP

Five healthy volunteers females aged 22 to 28 years were chosen They were not under the influence of drugs or other stimulants Their pupils were dilated to 8 mm or more with topical instillations of 0 5 % tropicamide and 10 % metaoxedrine Then after topical anaesthesia a scleral contact lens (for the tip of the recording electrode) (Fig 1) was applied to one of the eyes (Fig 2) It was equipped with two polyethylene tubes One was a saline agar bridge to the recording electrode and the other one filled with saline ended in a small beaker with saline the surface of which was placed 20 cm below the level of the eye In this way a gentle suction was applied keeping the contact lens still in relation to the eye Applanation tonometry before the application and immediately after the removal of the lens did not reveal significant changes in intraocular pressure

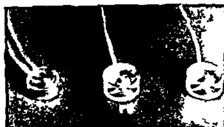


Fig 1

The contact lens (to the left) for the tip of the recording electrode and the two plastic chambers for the tips of the reference and ground electrodes respectively Saline bridges in agar filled polyethylene tubes lead to the different electrodes The contact lens is equipped with a second tube through which suction was applied.

Two plastic chambers (Fig 1) were placed on the forehead using pieces of ring shaped two sided adhesive tape (Fig 2) One was located 4 cm above the *cornea of the investigated eye and the other above the lateral canthus of the* other eye They served as holders for the tips of the saline agar bridges leading to the reference and ground electrodes respectively The chambers as well as the contact lens were filled with Methocel®



Fig 2

The contact lens and the two chambers attached to a volunteer

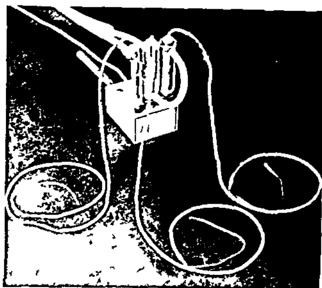


Fig 3

To the right two temperature stabilized matched calomel half cells used as recording and reference electrodes respectively The third calomel electrode mediates the ground connection All electrodes are equipped with saline bridges in agar filled polyethylene tubes

Matched calomel half cells that were temperature stabilized (± 0 1 C) by means of circulating water were used as recording and reference electrodes (Fig 3) The ground connection was also equipped with a calomel electrode The volunteer and the electrode system were shielded from alternating current etc by means of a wire net cage The potentials from the electrodes were fed into the differential inputs of a low drift d c amplifier They were lowpass filtered (220 Hz cut off 18 dB/octave) and reached a Tandberg digital tape recorder and a Hewlett Packard 5480 S signal analyzer where the measurements were displayed The d c drift of the electrode system was less than 15 μV The stability of the system was continuously checked on a separate oscilloscope Before registrations were started the eyes of the volunteers were exposed either to darkness or to a constant illumination for 80 min During the first 20 min of this period the contact lens etc was applied (In case of dark adaptation this latter procedure was carried out in an illumination of the eyes not exceeding 1 Lux) After the adaptation the base line was checked for stability during 10 min Then the illumination was suddenly changed either from darkness to 16

Lux or from 16 or 1100 Lux to darkness Only one change was used in the same experiment

Five sec recordings of the standing potential were carried out with 1 min intervals except during the first 5 min after the change in illumination when 30 sec intervals were used During recordings the volunteer was made to fix with her free eye upon a very weak deep red light in the ceiling In this way stable conditions were obtained within a few seconds this was checked on the separate oscilloscope Thereafter the measurement was read into the signal analyzer In a few experiments particularly designed to describe the initial fast change continuous recordings were made during 15 min This procedure required a great deal of co operation from the volunteer who kept her free eye closed

The average potential level obtained during the 10 min preceding the change in illumination is referred to as zero potential The SP amplitudes are given in mV above or below this level Fifteen experiments were carried out each re gistration lasting 50–60 min

In preliminary tests d c ERGs including the *a* and *b* waves were recorded in order to investigate a possible effect from suction Stimulus duration was 20 msec, flash interval 1 min and light intensity 3 5 relative log units above *b* wave threshold (The weakest light eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log rel intens 0) Using the suction contact lens the *a* and *b* wave amplitudes were first followed for 30 min without suction and then for 30 min with a negative pressure of 20 cm of water below atmospheric pressure inside the lens

Results

Fig 4 shows the results of a typical experiment where the effect on the SP of a sudden change in illumination from darkness to 16 Lux was studied after the stability of the base line had been checked for 10 min Following almost imme diately upon the change in light conditions a comparatively fast transient negative change was observed Thereafter a much slower variation of the SP in the form of damped oscillations was found It started in a positive direction building up a first peak of considerable amplitude. In Fig 5 the results from a similar experiment are illustrated

Fig 6 demonstrates the results from another representative experiment where the SP was continuously recorded in a very co operative volunteer who kept

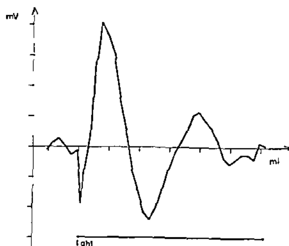


Fig 4
The results of a typical experiment showing SP oscillations in response to a change in illumination from darkness to 16 Lux SP recorded every min except from 0 to 5 min when 30 sec intervals were used Each point of the curve represents the average of two consecutive measurements

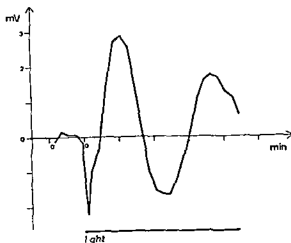


Fig 5
SP oscillations in response to a change in illumination from darkness to 16 Lux Recording procedure and plotting as for Fig 4



Fig 6
Continuous registration of SP changes following a change in illumination from darkness to 16 Lux

her eyes as still as possible without using a fixation light It may be noted that as a sign of stability the curve is very smooth This Figure depicts on an expanded time scale the first part of the SP variations after a change from darkness to 16 Lux The initial negative transient is easily seen as well as an indication of a small plateau where the slow oscillation has started but still seems to be influenced by the first fast component Thus also the fast component

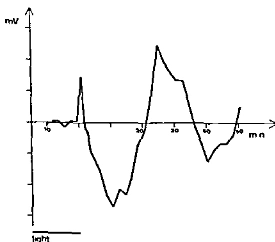


Fig 7
SP oscillations in response to a change in illumination from 1100 Lux to darkness Recording procedure and plotting as for Fig 4

appears to be cyclic in nature Since after the first few minutes no evidence of fast variations superimposed upon the slow oscillation is seen the former change seems to be damped very quickly

Fig 7 illustrates the effect of a sudden change from 1100 Lux to darkness in a typical registration The oscillatory amplitudes are smaller than they were when illumination was changed in the opposite direction although this latter step was only 16 Lux However the curve is more or less a mirror image of those in Figs 4 and 5 Thus the initial rather fast transient change is positive *and the slower oscillation begins in a negative direction In Fig 8 the results* from a similar experiment are illustrated

The other experiments confirmed the above findings

The maximum amplitude of the comparatively fast positive or negative transient occurred 0 5–1 0 min after the change in illumination The frequency of the slower oscillations was about 2/hour and the full amplitude of the first oscillation was of the order of 5mV when the illumination was changed from *darkness to 16 Lux and 2mV in response to a change from 1100 Lux to darkness* When the illumination was altered from 16 Lux to darkness the amplitude was even smaller In some of the experiments the oscillations seemed to be superimposed upon a small and much slower change

To test the influence of the suction contact lens the *a* and *b* waves were followed for 30 min without suction and then for another 30 min with suction The *a*-wave was measured from the base line and the *b* wave from the trough of the *a*-wave No significant effects of the suction on these ERG components were noted

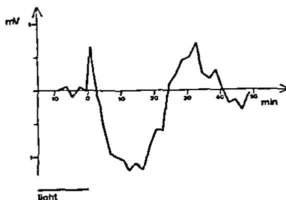


Fig 8
SP oscillations in response to a change in illumination from 1100 Lux to darkness Recording procedure and plotting as for Fig 4

Discussion

Earlier attempts to follow the SP were often disturbed by considerable d c drift which to a great extent could have been avoided by the use of calomel electrodes Changes at the electrode – animal junctions also caused problems (Yoshida 1953 Muller Limmroth 1954) Gouras & Carr (1964) using 4/sec flicker stimuli were able to follow the SP in the monkey for about 1 hour and demonstrated 2/hour oscillations Slow SP variations with a somewhat different time course in different species were found also by Kikawada (1968) but the experiments were not long enough to establish clearly the exact characteristics

In the present study the SP was found to vary like a damped oscillation in response to a sudden change in the illumination of the eye The timing and the general shape of these directly measured SP oscillations were in close agreement with the indirectly studied SP changes (EOG variations) after sudden light and dark steps (Kris 1958 Kolder 1959 Taumer 1974 and others) This applied both to the initial fast damped variation within the first few min and to the slower damped oscillations with a frequency of about 2/hour The latter were very small after 1 hour and should be negligable after 80 min For this reason the eyes of the volunteer of the present study were kept in constant illumination for 60–80 min before registrations were started A true description of the initial transient was obtained only in the experiments where continuous registration was performed The magnitude of the oscillations is evident from the Figures but more precise statistics about the amplitudes in relation to different light changes will be available when a larger material has been collected

An attempt to follow the SP directly with standard ERG equipment (a c amplifier and standard contact lens electrode) was made by Sole Alfieri & Dumas Perrier (1971) However the variations in polarizing currents of ordinary ERG electrodes are too large to permit a true registration of the SP changes Moreover the usual contact lenses slide on the eye surface causing too much potential variation for long term SP studies The present technique included d c amplification a suction contact lens and very stable matched calomel electrodes the temperature of which was kept constant The d c drift of the electrode system continuously followed for 1 hour did not exceed 15 μV On the other hand it cannot be fully excluded that the electrode – corneal and electrode – forehead junctions change their properties during a long term registration In some experiments the 2/hour oscillations seemed to be superimposed upon a small and much slower variation The exact nature of the latter cannot be definitely determined on the basis of the present experiments However in this connection it may be noted that the 2/hour oscillations of the *c* wave amplitude were convincingly shown to be superimposed upon slower 0 5 hour oscillations (Calissen

dorff Knave & Persson 1974) (sheep) This is interesting since the *c* wave and the SP are derived at least partly from the same source (see introduction) Very slow EOG variations were described by Davis & Shackel (1960) and by Jacobs Feldman Rabinowitz & Bender (1973)

An alteration in SP in response to a change in intraocular pressure (IOP) was described by Stepanik (1958) who used a special suction contact lens with strong negative pressures (40–300 mmHg below atmospheric pressure) to provoke a rise in IOP No significant changes were noted while the IOP was rising but an increase in SP occurred when the suction was discontinued The *b* wave is known to be sensitive to an increase in IOP (Wulfing 1963 Henkes Usami & van Lith 1968) In the present study a very moderate negative pressure of about 15 mmHg below atmospheric pressure was applied This suction did not affect the *b* wave Applanation tonometry did not reveal any significant changes in IOP before and after the experiments A pilot study also showed that not even a suction of 20 mmHg influenced the IOP Thus there is no reason to believe that the slight suction used should exert any influence on the SP either

Until the effects of electrical shunting in the body tissues a somewhat variable positioning of the reference electrode and perhaps also changes at the electrode – body junctions have been evaluated the SP should not be given in absolute values In the EOG technique the light peak/dark trough ratio is generally used as the main parameter instead of the EOG potential (Arden & Kelsey 1962)

For a better understanding of the relationship between the c wave of the ERG and the SP it should be valuable to carry out simultaneous direct d c recordings of these potentials The present technique used on human volunteers allows this kind of comparative experiment already in progress (Nilsson & Skoog 1974) Since a true registration of the *c* wave requires very stable conditions it seems unrealistic to combine the conventional EOG procedure with simultaneous *c* wave recordings

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The Annual Meeting of the German Ophthalmological Society will be held from September 11 through 24 1975 at the Haus der Technik Essen Germany The main subject is *Peripheral Retina* Simultaneous translation in German English and French For additional information write to Prof Meyer Schwickerath D 43 Essen Universitats Augenklinik President of the Society or Prof Jaeger permanent Secretary of the Society

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ACTA OPHTHALMOLOGICA VOL. 53 1975

Dedicated to Sigurd Ry Andersen
on the Occasion of his Sixtieth Birthday March 22 1975

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THE VALUE OF AN OPHTHALMIC TUMOUR CENTRE

BY

E GREGERSEN

An account is given of the clinician's experience of an ophthalmic tumour centre established in an institute of ophthalmic pathology serving the whole of Denmark (population 5 mill) The Tumour Centre performs supplementary preoperative examination of patients and gives advice with respect to biopsy attempted total extirpation enucleation or observation The activities of the tumour centre have proved extremely valuable as it contributes to establishing more general lines concerning biopsy attempted total extirpation observation, or enucleation, to the benefit of patients as well as research It is a presupposition that the advisory activity is carried out as an intimate collaboration between ophthalmic pathology and clinical ophthalmology

Key words ophthalmic tumour centre – attempted total extirpation – biopsy – enucleation – observation.

In the Institute of Ophthalmic Pathology S Ry Andersen M D founded the Danish Ophthalmic Tumour Centre ten years ago as a consultative service with the aim of giving a supplementary evaluation of all patients for whom biopsy tumour removal or enucleation are considered The background for establishing such a clinical activity in an institute of ophthalmic pathology was chiefly that

two of the doctors of the Institute (S Ry Andersen and O A Jensen) are pathologists as well as ophthalmologists

As the Institute of Ophthalmic Pathology and its Tumour Centre are housed in Rigshospitalet it is only natural that the Eye Department of this hospital has been the biggest customer In recent years except for the more commonplace conditions the Eye Department of Rigshospitalet has been sending all patients to the Tumour Centre for a supplementary preoperative clinical evaluation regarding extirpation or biopsy of tumours of the eyelid conjunctiva cornea and orbit Similarly all cases of intraocular lesions suspected of malignancy have been referred for preoperative assessment To give some idea of the size of the activity in 1973 the Eye Department referred a total of 70 patients to the Tumour Centre – 12 with palpebral 12 with conjunctival 6 with corneal 9 with orbital and 31 with intraocular tumours

As the Tumour Centre receives patients from the whole of Denmark a wide experience and expert knowledge is accumulated in regard to the optimal application of observation biopsy extirpation or enucleation The Tumour Centre also takes part in following up the patients when observation has been decided upon Preoperative assessment by the ophthalmic pathologists in the Tumour Centre also has the advantage that the technique as well as the site of biopsy can be adapted to the clinician as well as to the ophthalmic pathologist

The Danish Ophthalmic Tumour Centre has come to stay – and to grow It contributes to establishing more general lines throughout the country in the often difficult questions of biopsy attempted total extirpation or observation e g in patients with conjuncival or palpebral naevi or melanomas As regards observation versus enucleation in cases of intraocular lesions suspected of malignancy it is evident that a tumour centre serving an entire country can improve the evaluation and promote treatment as well as research

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Dedicated to Sigurd Ry Andersen
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Ullevål Hospital Departments of Pathology and Ophthalmology
(Heads Professor Kristen Arnesen and Professor Jan Ytteborg)
University of Oslo Norway

MALIGNANT MELANOMA OF THE CHOROID AS RELATED TO COEXISTENT BENIGN NEVUS

BY

KRISTEN ARNESEN and MAY NORNES

Ninety five cases of malignant choroidal melanoma collected during a 15 year period have been studied with special emphasis on histological type coexistence of benign nevus and prognosis Elements of benign nevus were found in 78 % of the cases The data tend to support the hypothesis that most cases of malignant choroidal melanoma have their origin in a preexisting benign nevus and that the tumours undergo a gradual change from a differentiated to a less differentiated type

Key words choroid – malignant melanoma – benign nevus

In the eight year period 1953–60 Mork (1961) found 220 new cases of malignant melanoma of the eye in Norway This figure included the conjunctival melanomas whose number is small in relation to the uveal or choroidal melanomas

In the 10 year period 1960–69 279 new cases of intrabulbar malignant melanomas were registered (Pedersen 1974) The population of Norway was 3 75

Received December 3 1974

million in 1966 and the average annual incidence of malignant uveal melanoma was thus about 0 75 per 100 000 which corresponds well to the figure given by Jensen (1970) for Denmark but is only a little more than half the incidence estimated by Ganley & Comstock (1973) for Washington County Maryland

According to the available data no significant change has taken place regarding the incidence of this tumour during the last 20 years in Norway

The present study comprises a material of malignant melanomas of the choroid studied histopathologically at the Department of Pathology Ullevål Hospital from 1960 to the fall of 1974 The main purpose was to investigate how frequently elements of a benign nevus occur at the basis of the malignant melanomas and to try to find out whether the coexistence of a benign nevus has any influence upon the prognosis In addition a few other characteristics of the material has been studied both from the clinical and the pathological point of view

Material and Methods

Ninety five enucleated eyes with a clinical and pathological diagnosis of malignant choroidal melanoma were studied from 1960 till October 1974 Sixty five cases were treated in the Department of Ophthalmology Ullevål Hospital and 30 eyes had been enucleated in various eye clinics outside Oslo and a few larger cities Thus the Ullevål material was taken from an urban population whereas the rest of the material came from smaller towns and rural districts where the accessibility of ophthalmologists is not as easy as in the capital

The enucleated eyes were fixed in 4 % formaldehyde solution and the cups were cut in the pathology laboratory after at least 48 hours fixation Ten to twenty routine sections were cut from each eye through the central part of the tumour and in most cases through the optic disc The sections were stained with hematoxylin erythrosin saffran Since 1971 the protocol of the laboratory of the *Ophthalmic Branch of the Armed Forces Institute of Pathology* has been strictly adhered to

Histologically the tumours were typed according to Callender s classification Because the material is small it was found practical to operate with only two classes for the statistical evaluation viz the spindle cell type and *other types*

The coexistence of benign nevus was ascertained through the finding of at least 3–4 layers of cells along the base of the tumour having the characteristic features of cells from a benign choroidal nevus as described by Naumann (1970)

and differing morphologically from the cells of the main bulk of the tumour (Figs 1 and 2)

The prognosis was studied in 49 cases covering the period from 1960 up to and including June 1969 allowing an estimation of the 5 year mortality rate The follow up study has been made possible through the kind cooperation of Dr Einar Pedersen Director of The Cancer Registry of Norway

For the statistical evaluation *Student's t* test and *X* analysis have been used

Results

In the 10 year period from 1960–69 60 cases of malignant choroidal melanoma were studied histopathologically in our laboratory This number includes the 49 cases available for the prognosis study plus the cases studied in the second half of 1969 and a few cases which could not be traced by The Cancer Registry for prognosis study The material amounted to 22 % of all cases of malignant choroidal melanoma in Norway in this period The number of cases treated in the Department of Ophthalmology at Ullevål in the same period was 40 or 14.3 % of the national material which corresponds closely with the relative size of the population in Oslo

Sex and age distribution

In the whole material from 1960–74 there were 52 men and 43 women giving a sex ratio of 1 21 (Table I) In the Ullevål clinical material the ratio was 1 03 *versus* 1 73 for the country material

The youngest patient was 25 and the oldest 88 years The mean age at enucleation was approximately 61 ± 12 years – about 2 years higher for women than for men both in the Ullevål and the country material For both sexes the mean age was slightly higher in the country material but none of these differences were significant

In the period 1960–68 there were 47 cases and in the following period 1969–74 48 cases were recorded In the first period the mean age at enucleation was 4 years higher than in the second period and this difference approaches the lowest significance level (Table II)

Table I
Malignant melanoma of choroid 1960–74 Histological types and sex distribution

	Men	Women	Total	Sex ratio (men to women)
Spindle cell	24	29	53	0 83
Other types	28	14	42	2 00
All types	52	43	95	1 21

X = 4 32 $P < 0\ 05$*

Table II
Malignant melanoma of choroid 1960–68 *versus* 1969–74 Mean age at enucleation

	Number	*Mean age*	Standard deviation
1960–68	47	63 28	12 12
1969–74	48	59 00	12 46
1960–74	95	61 14	12 42

$t = 1\ 7$ $0\ 1 > P > 0\ 05$*

Histological type

The distribution of the two main histological types is presented in Table I In women there is a significant preponderance of the spindle cell type over other types in relation to men When the material is divided into the 1960–68 and the 1969–74 periods the significance level is of the same order for the first period ($P < 0\ 05$) whereas for the second period there is no significant difference in the distribution of the histological types in the two sexes (Table III)

Table III
Malignant melanoma of choroid Histological types in two observation periods in relation to sex

	Men	Women	Sex ratio	*P*
1960–63				
Spindle cell	10	15	0 67	
Other types	16	6	2 67	< 0 05
1969–74				
Spindle cell	14	14	1 00	
Other types	12	8	1.50	> 0.50

The mean age was 4 years lower in the spindle cell type than in other types (Table IV) but this difference is not significant

Table V shows the distribution of the main histological types in relation to the groups of patients under and over 60 years of age In younger patients the spindle cell type predominates whereas in patients over 60 years there is a slight predominance of other types

Table IV
Malignant melanoma of choroid 1960–74 Mean age at enucleation in relation to histological types

	Number	Mean age	Standard deviation
Spindle cell	53	59 40	12 90
Other types	42	63 33	11 43
All types	95	61 14	12 42

$t = 1.57$ $P > 0 1$ n s

Table 3
Malignant melanoma of choroid 1960–74
Histological types in relation to age groups
under and over 60 years

	← 60 yrs →		Total
Spindle cell	29	24	53
Other types	14	28	42
All types	43	52	95

$\chi^2 = 4.39$ $\quad P < 0.05$*

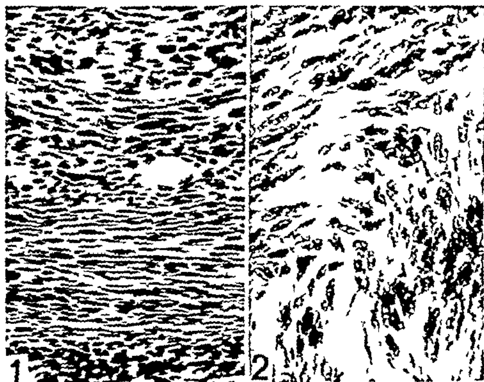


Fig 1
Eye No 10987/74 Malignant melanoma of choroid Elements of benign nevus along the basis of the tumour In the upper part transition to malignant melanoma × 435

Fig 2
Same as Fig 1 The picture is taken from the main bulk of the tumour which was typed as a spindle cell B tumour × 435

Table VI
Malignant melanoma of choroid 1960–74
Coexistence of benign choroidal nevus

	Nevus +	Nevus –	Total
Men	39 (75 %)	13	52
Women	35 (81 %)	8	43
Total	74 (78 %)	21	95

The sex difference is not significant.

Coexistence of benign nevus

The coexistence of a benign nevus and a malignant melanoma is depicted in Figs 1–2 The nevus cells clearly differ from the malignant melanoma cells being mostly spindle shaped with small nuclei with condensed chromatin The cells are orderly arranged in parallel bundles occupying the outer part of the choroid where they can be found in close relation to ciliary nerves as pointed out by Vogel (1970) There is usually a gradual transition from the outer part of the nevus to the overlying malignant melanoma with a zone of intermediate cell types The nevus elements can be recognized all the way along the basis of the

Table VII
Malignant melanoma of choroid in observation periods 1960–68 and 1969–74 Coexistence of benign nevus

	Nevus +	Nevus –	Total
1960–68	34 (69 %)	15	49
1969–74	40 (87 %)	6	46
1960–74	74 (78 %)	21	95

$\chi^2 = 4.25$ $P < 0.05$

Table VIII
Malignant melanoma of choroid 1960–74 Coexistence of benign nevus in relation to histological types

	Nevus +	Nevus –	Total
Spindle cell	44 (83 %)	9	53
Other types	30 (71 %)	12	42
All types	74 (78 %)	21	95

$\chi^2 = 1.83$ $P > 0.10$ n s

malignant melanoma or in scattered foci often very marked along the peripheral attachment of the tumour to the choroid

Coexistence of a benign nevus was found in 74 of the 95 cases of malignant melanoma or 78 % There was a slight preponderance in women but the difference is not significant (Table VI)

There was no difference between patients under and over 60 years of age as to the coexistence of a benign nevus and even in the small group of seven patients under 50 years the relative occurrence of a benign nevus in relation to the malignant melanoma was of the same order of size as in the whole group (5 out of 7)

In the 5 year period 1969–74 the coexistence of benign nevus and malignant melanoma was recorded more frequently than in the preceding 9 year period (Table VII)

The malignant melanomas of the spindle cell type reveal a greater tendency to include elements of a benign nevus than do the other histological types (Table VIII)

Prognosis

Forty nine patients are included in the prognosis study covering the period from 1960 to 30 June 1969 The total number of deaths and number of deaths caused by metastases from malignant melanoma are accounted for according to the information provided by The Cancer Registry of Norway

Table IX

Malignant melanoma of choroid 1960–69 Prognosis in 5 year observation period

	Alive 5 yrs after enucleation	Died of metastases within 5 yrs	Died of other causes	Total
Men	18	7	2	27
Women	11	7	4	22
Total	29	14	6	49

The sex difference in specific mortality rate is not significant

The total number of deaths was 20 Fourteen patients had died of melanoma metastases Thus the total 5 year mortality rate was 41 % and the specific mortality rate 29 %

The 5 year survival and mortality data are presented in Table IX Even if more men than women survived for 5 years there is no established sex difference

Table X

Malignant melanoma of choroid 1960–69 Mean age at enucleation in relation to the prognosis in a 5 year observation period

	Number	Mean age [illegible]	Standard deviation
Alive 5 yrs after enucleation	29	58.69	12.44
Died within 5 yrs of metastases	14	68.99	9.24

$t = 2.84$ $P < 0.01$ *

Table XI
Malignant melanoma of choroid 1960–69
Prognosis in relation to histological type

	Alive after 5 yrs	Died of metastases within 5 yrs	Total
Spindle cell	16	5	21
Other types	13	9	22
	29	14	43

$\chi^2 = 1.43$ $P > 0.20$ n.s.

The patients who were alive 5 years after enucleation of one eye for malignant melanoma were significantly younger at enucleation than those who died of metastases within 5 years (Table X).

There was a higher survival rate in the spindle cell group than among patients with less differentiated tumours (Table XI). This difference which is not sig

Table XII
Malignant melanoma of choroid 1960–69
Prognosis in relation to age groups under and over 60 years at enucleation

	Alive after 5 yrs	Died of metastases within 5 yrs	Total
Under 60 yrs	17	2	19
Over 60 yrs	12	12	24
All ages	29	14	43

$\chi^2 = 7.53$ $P < 0.01$ *

Table XIII
Malignant melanoma of choroid 1960–69 Prognosis in relation to coexistence of benign nevus

	Nevus +	Nevus –	Total
Alive 5 yrs after enucleation	22	7	29
Died within 5 yrs of metastases	7	7	14
	29	14	43

$\chi^2 = 2\,89$ $0\,10 > P > 0\,05$*

nificant may partly be accounted for by the fact that patients with spindle cell tumours tend to be younger than those with other histological types (Table IV)

The 5 year melanoma mortality rate was significantly lower in patients under 60 years of age at enucleation than in older patients (Table XII) Among seven patients who were less than 50 years old at enucleation one died after 6 years of carcinoma of the colon The others were alive in July 1974 from 5 to 14 years after enucleation

The prognosis tended to be better when elements of a benign nevus were found at the base of the tumour than when these were not present (Table XIII)

Discussion

In this material there is a slight preponderancy of men as was also found by Mork (1961) and Jensen (1963) in their studies from Norway and Denmark respectively

The mean age at enucleation is higher in our material (Table II) than in Mork s and Jensen s We can offer no clear explanation of this fact The lower mean age in 1969–74 compared with 1960–68 can probably be explained by the easier accessibility of ophthalmologists and perhaps by a more active attitude towards suspected intrabulbar *neoplasms*

We have classified 56 % of our tumours as belonging to the spindle cell type (Table I) whereas Jensen (1963) found that 37 % of his cases belonged to this group This discrepancy probably reflects varying criteria for typing

In our material the spindle cell type predominates over other types among women (Tables I and III) In Jensen s material (1963) the spindle cell types had a better prognosis than other types We should therefore expect a better prognosis for women in our material which however is not the case (Table IX) This again we can not explain

The spindle cell cases tend to be younger than those belonging to other cell types (Tables IV and V) This may indicate that the histological type is not a stationary parameter but changes from a higher to a lower degree of differentiation with the passing of time

Elements of a coexisting benign nevus were found in 78 % of the eyes in the present material (Table VI) This observation is in good accordance with that of Yanoff & Zimmerman (1967) who found benign appearing nevus cells within or along the edges of malignant choroidal melanomas in 73 out of 100 cases

The relationship between benign nevi and malignant melanomas of the uvea has been studied by many workers (Houwer 1928 Albers 1940 Naumann Yanoff & Zimmerman 1966 Yanoff & Zimmerman 1967 Vogel 1970 Ganley & Comstock 1973) There are different concepts as to the true nature of the benign pigmented lesions of the uvea (Naumann 1970 Dhermy & Limon 1972) and their proper designation With Naumann (1970) we prefer to use the non committal name *benign nevus* and avoid the term *benign melanoma* To Scandinavian workers *melanoma* is an ominous word being often used synonymously with *malignant melanoma*

Benign nevi of the choroid are relatively common In an autopsy material Naumann (1970) found benign nevi in 11 out of 200 eyes which means that about 10 % of human beings (in Hamburg) harbour a benign nevus in one eye and he concluded that most malignant choroidal melanomas arise from pre existing benign nevi The same opinion was held by Yanoff & Zimmerman (1967)

On the basis of our own findings we want to support the conclusion that there is a close connection between benign nevi of the choroid and the development of malignant melanomas even if only a small number of benign nevi develop into a malignant neoplasm with potential metastases

We also think that there may be a continuous and gradual change from benign nevus to spindle cell malignant melanoma to less differentiated melanomas of the choroid

The observations which are condensed in Tables II VII and VIII tend to support the above postulation In the period 1969–74 the mean age at enuclea

tion for malignant choroidal melanoma was lower than in the preceding period Correspondingly the percentage of cases showing elements of a benign nevus had increased from the earlier to the recent period and elements of benign nevi tended to be associated more often with the spindle cell type than with the other types

The prognosis in the 5 year observation group of 49 patients is in fairly good accordance with Jensen s data (1963) He found that 37 % of the total material had died of metastases within 5 years Our corresponding figure is 29 % (14 out of 49 cases) The difference may be explained by a relatively higher mortality from other causes in our material

Patients who were alive 5 years after enucleation were 10 years younger at enucleation than those who died of metastases within 5 years (Table X) A similar trend can be read from Table XII and it may be concluded that the prognosis of malignant choroidal melanoma is better for younger patients than for older

Our data accord with the well known fact that melanomas of spindle cell type have a better prognosis than those of less differentiated types (Table XI) and they indicate that the coexistence of a benign nevus is a favourable prognostic sign (Table XIII)

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Dedicated to Sigurd Ry Andersen
on the Occasion of his Sixtieth Birthday March 22 1975

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BILATERAL MASSIVE GLIOSIS OF THE RETINA INVOLVING THE OPTIC NERVES

Report of a Case

BY

ROBERT Y BARISHAK and R STEIN

A 40 year old woman blind in her right eye since childhood and in the left since the age of 15 presented a gliomatous tumoral mass filling up the eyes completely Sections of the optic nerve of one eye showed the same cellular aspect noted in both intraocular tumors The optic foramina had a normal size and there were no signs of Recklinghausen s disease The diagnosis was bilateral reactive gliosis but the presence of a congenital anomaly has to be postulated in order to explain the involvement of the optic nerve in the process of intraocular gliosis

Key words retina – optic nerve – gliosis

Gliomas of the optic nerve are slowly growing tumors which usually arise in the orbital segment of the nerve They may extend forwards and protrude into the vitreal cavity at the region of the optic disc or backwards towards the chiasm and provoke an enlargement of the optic canal (Reese 1963) Bilaterality has been rarely observed (Goldsmith 1949 and Saran & Winter 1967) Histologically the diagnosis may be quite difficult in view of the similarity to reactive gliosis

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of the retina (Yanoff Zimmerman & Davis 1971) It becomes a problem to decide when an intraocular tumor composed of glial cells should be called glioma and when it should be called gliosis especially when the optic nerve shows signs of involvement and has the same cellular aspect as the intraocular tumor

Case report

A 40 year old white woman presented in the Outpatient Department with huge corneal staphylomata nystagmus and amaurosis in both eyes The family history has been non contributory She had been blind in her right eye since her early childhood In the left eye vision started to deteriorate at the age of 12 and was nil at the age of 15 Since then she experienced from time to time attacks of headaches and pain particularly in the right eye About 6 months after her first visit to the Outpatient Department she was admitted to hospital with a traumatic rupture of the left corneal staphyloma A bloody secretion was seeping from the perforation The eye was enucleated It was of an irregular cylindrical shape and measured 30 × 17 × 17 mm From the staphylomatous



Fig 1a

Left eye cut meridionally Intraocular tumor mass protrudes anteriorly out of the globe

Fig 1b

Right eye cut horizontally Huge anterior staphyloma Retrobulbar tumor mass connected with its intraocular extension



Fig 2
Spindle cells in palisade arrangement 300 x

cornea only a narrow rim around the limbus remained A hard potato like mass was protruding through the perforation At the posterior pole, the optic nerve and rim of sclera around it were missing having been left behind by the surgeon Fig 1 a shows the globe after it had been cut meridionally

Histological examination revealed that the mass occupying the whole interior of the globe and protruding outwards was composed of spindle cells with nucleolated nuclei in a palisade and perivascular arrangement (Fig 2) In some places the arrangement of cells and fibers resembled Verrocay bodies in others a microcystic degeneration was seen imparting a more areolar appearance (Fig 3) Other less pertinent secondary findings were a severe uveitis ossification of the choroid destruction of the cornea aphakia and a severe inflammatory reaction at the limbus in the episclera and posterior sclera

The tumor was diagnosed as a neurilemoma General examination including a search for *cafe au lait* spots of the skin, was negative for Recklinghausen s disease The right eye was not exophthalmic and its huge staphyloma did not have the appearance of a microphthalmus with cyst. X ray examination of the skull showed that both optic foramina were of normal size and shape One week after the operation the conjunctival sutures inside the socket gave way and whitish friable material emerged The socket was cleaned from all this material and the conjunctiva resutured

Eight months later the patient returned because of intolerable pains in her right eye and demanded enucleation. At the time of enucleation a hard sausage like mass was encountered at the posterior pole which could be cut only with difficulty A part of the

of the retina (Yanoff, Zimmerman & Davis 1971). It becomes a problem to decide when an intraocular tumor composed of glial cells should be called glioma and when it should be called gliosis, especially when the optic nerve shows signs of involvement and has the same cellular aspect as the intraocular tumor.

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Fig. 1 a

Left eye cut meridionally. Intraocular tumor mass protrudes anteriorly out of the globe.

Fig. 1 b

Right eye cut horizontally. Huge anterior staphyloma. Retrobulbar tumor mass connected with its intraocular extension.

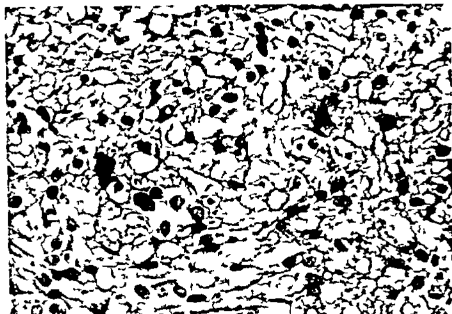


Fig 5

Section through the right optic nerve Most of the cells are of the astrocytic type 600 x

mass had remained in the orbit and was removed separately The eyeball with the outward protruding mass looked like a gross rounded sledge hammer with its huge head directed forward The globe itself measured only about 12 mm in diameter but superimposed on it was this staphyloma like head of 25 mm in diameter and 15 mm thick It was covered with a flat thin opacified cornea and thin sclera (Fig 1 b)

Microscopically the mass filling the intraocular cavity was composed of long hair like fusiform cells in fascicular and in some places perivascular arrangement (Fig 4) At other sites cystoid spaces prevailed This aspect was identical to that of the intraocular tumor found in the left eye

Sections of the optic nerve behind the tumoral mass showed the same areolar appearance noted in the intraocular tumor of both eyes (Fig 5)

At the time our histological diagnosis was glioma of the optic nerve of the astrocytic type which had penetrated into the ocular cavity and filled it completely In reviewing our slides of the first enucleated left eye and repeating the Holzer stain for the identification of glial cells we came to the conclusion that this eye also harboured a gliomatous tumor which probably originated from the optic nerve and disc which were missing in the specimen and had grown into the vitreal cavity and filled up the eye But the bilaterality of the process and the normal size of the optic foramina mitigated against this diagnosis

Fig 3
Cystic spaces within the tumor 500 x

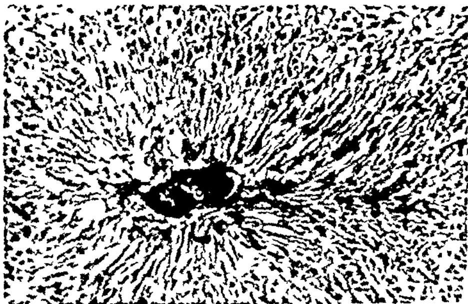


Fig 4
Palisade and perivascular arrangement of fusiform cells 500

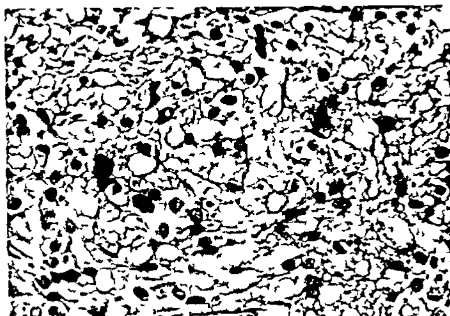


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The blocks of both specimens were sent to the Eye Pathology Laboratory of the U C Medical Center San Francisco California and the slides reviewed by Dr W Spencer Dr M Hogan and later on by Dr L E Zimmerman Dr S Ry Andersen and Dr O A Jensen Their diagnosis was bilateral massive gliosis also involving the optic nerve But to explain the involvement of the optic nerve in this process of gliosis they had to suggest the presence of a congenital anomaly possibly a failure of complete closure of the fetal fissure permitting involvement of both the optic nerve and the globe Our slides did not reveal any sign suggesting such a possibility

Comments

This case illustrates the difficulties that one may encounter in the diagnosis of intraocular glial tumors When the first enucleated eye was studied histologically the diagnosis was neurilemoma we had no segment of the optic nerve on which to evaluate the relationship of the intraocular tumor with the nerve The second eye was sent with a stump of the optic nerve the same cellular aspect was noted in the second eye as in the first Moreover the same areolar aspect observed in the intraocular tumor was noted in the sections of the optic nerve This led us to the diagnosis of glioma of the optic nerve which penetrated into the vitreal cavity and filled up both eyes completely The severe uveitis the intraocular ossification and other signs of inflammation were attributed to the long standing of the intraocular tumor The whitish material which came out of the socket of the first enucleated eye was considered retrospectively as remnants of gliomata But the bilaterality of the tumor (a rarity in gliomas of the optic nerve) and the presence of optic foramina of normal size mitigated against the diagnosis of intraocular glioma On the other hand the areolar appearance in sections of the optic nerve strongly favored an optic nerve origin of the intraocular tumor

In conclusion we might consider this case to be one of bilateral intraocular massive gliosis but not without assuming the presence of an anomaly in both eyes as the one mentioned above to explain the involvement of the optic nerve in this process of gliosis

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MALIGNANT MELANOMA OF THE CHOROID IN AN 11 MONTH OLD INFANT

BY

HANS FLEDELIUS and ANNA MARIE LAND

A malignant melanoma of the choroid was observed in an 11 month old fair skinned Danish boy – one of the youngest cases ever published There was no evidence of pre existing melanosis of eyes or skin. The tumour showed histopathologically a typical adult pattern and was classified as a mixed tumour The melanin content was marked
In some earlier reports on uveal tumours within the first year of life pigmentation was sparse or lacking a hypothesis has therefore previously been advanced That melanomas are unable to produce melanin so early in life The present case renders proof against this hypothesis

Key words ocular tumours in infancy – malignant melanoma of the choroid – congenital melanoma

Malignant melanomas of the uvea are extremely rare in early infancy and the diagnosis can be questioned in some of the previously published cases because of atypical microscopic patterns This report deals with a case of malignant melanoma of the choroid verified histopathologically in a Danish boy aged only 11 months

Received December 4 19 4

Material and Methods

Clinical history (Eye Dept Odense Sygehus rec no 180373) The patient was an 11 month old boy with a red eye of 2 weeks duration The family history was negative as regards tumours of eyes and skin

He was number two of two Pregnancy and delivery were uncomplicated, birth weight 3 500 g growth and development were normal About the age of 6 months he had suffered from an exanthematous disease (not further classified) for a few days Otherwise he had been healthy

On a tentative diagnosis of conjunctivitis the general practitioner prescribed topical treatment with antibiotics (Chloramphenicol) but without clinical improvement After 2 weeks the boy was seen by an ophthalmologist who immediately transferred him to the University Eye Clinic of Odense because of tumour of right eye pupil non reacting to light

On admission the boy seemed in good general health Hyperpigmentation was not noted especially not in the iris of the diseased right eye or in the conjunctiva and surrounding skin. The left eye was entirely normal

Examination of the *right eye* (in general anaesthesia) Congestion of conjunctival episcleral and ciliary vessels cornea transparent Congested iris vessels were visible in the inferior part and the anterior chamber was shallow in the corresponding sector Gonioscopy inconclusive tumour tissue did not occur in the chamber angle. Ophthalmoscopically a solid mass with a grey red surface was seen in the lower quadrants of the eye The retina was detached also in the upper part of the eye so as to hide the optic disc The solid mass gave a shadow on transillumination

The eye was enucleated on the boy s first anniversary

When examined 6 months later the boy was still in perfect health There was no evidence of local recurrence or metastases as judged from general examination and laboratory tests X ray of the chest was normal

Pathology (Øjenpat Inst no 142/74) The eyeball was fixed in buffered 4 % formaldehyde and 1 % glutaraldehyde (pH 7 2) for 24 hours It was measured in three dimensions examined in a dissecting microscope and cut obliquely Routine paraffin technique was applied Deparaffinized hydrated sections were stained with haematoxylin eosin, haematoxylin phloxine safranin Masson Fontana s stain for melanin and iron stain Depigmentation with sodium permanganate was carried out

Results

The size of the eyeball (22 x 23 x 23 mm) and the outer surface were normal

In the cut eyeball (Fig 1) a heavily pigmented tumour was seen inferiorly occupying about half of the vitreous space The retina was totally detached The solid mass came close to the optic nerve anteriorly it extended to the iris root and the posterior lens surface

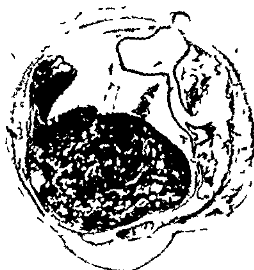


Fig. 1
Survey of the eyeball showing the large pigmented tumour with vascular spaces. The retina is totally detached. Haematoxylin eosin (× 3).

Microscopical examination showed a tumour with origin in the choroid. Invasion of the sclera, the optic nerve and the iris (Fig. 2) was not observed. The tumour was moderately pigmented and heavily vascularized. The tumour cells (Fig. 3) were partly spindle shaped, partly epitheloid; there were multinucleated giant cells with prominent nucleoli but only few mitoses. Tumour cells were seen within the lumina of some of the large vascular spaces. The stromal tissue was sparse with scattered old and recent haemorrhages.

Masson Fontana, iron stain and depigmentation showed the intracytoplasmatic granules to react like melanin.

Histopathological diagnosis: Malignant melanoma of the choroid, mixed cell type, predominantly epitheloid.



Fig 2

The anterior part of the tumour invading the ciliary body Displaced ciliary processes are seen The chamber angle structures are uninvolved Haematoxylin eosin (100)



Fig 3

The cellular pattern of the tumour Spindle shaped and more anaplastic (epitheloid) cells are seen intermingled. There is marked pleomorph sm Haematoxylin eosin (x 275)

Discussion

Melanomas of the choroid and ciliary body – the most frequent primary malignant tumour of the eye – are extremely rare within the first years of life. According to Callender et al. (1942) and Paul et al. (1962) only one out of thousand malignant intraocular melanomas belongs to the age group 0–9 years. In Denmark Jensen (1963) found one case – a boy aged 7 years – in a 10 year material (1943–1952) comprising a total of 295 malignant melanomas of the choroid and ciliary body. *Malignant melanomas have since then been registered on a national* scale in Denmark and the present case is the first one occurring in infancy since the boy in Jensen's material. This accords with the expectancy rate of 1–2 per 1 000 intraocular melanomas. Every century four to five infantile cases should be expected in Denmark.

From the above it is evident that the literature on malignant uveal melanomas *in infancy is concerned mostly with single cases. Such reports have recently been* published by Jones (1967), Chaves & Cranville (1972), Newman & Wolter (1973) and Scheffer et al. (1974). More extensive reviews were given by Cury et al. (1959), Apt (1962) and Verdaguer (1965). It appears that the malignant cases in children clinically and histopathologically behave like the intraocular melanomas in adults. With all uveal melanomas taken into consideration a *difference however occurs between young and adult series. As regards the site* of origin a relative preponderance of iris melanomas has been found in the age classes below 20 years.

Two points are worth further discussion: a) the validity of the microscopic diagnosis and b) the time of onset of the tumour.

a) As to the histologic appearance some of the published early cases seem to be "sarcomas" – without the typical features of malignant uveal melanomas. Thus the tumour reported in a 7 month old child by Burki (1961) had only very few melanin deposits; it was microscopically classified as a sarcoma, but the *author left open the possibility of a non pigmented malignant melanoma. Sup*port for this view was made up from a series of Badtke & Zimmermann (1956); they emphasized the lacking or incomplete formation of pigment so early in life. Malignant uveal melanomas were therefore considered histologically to have somewhat different "equivalents" in the first year(s) of life.

b) A series of congenital benign melanomas was recently reported by Reese (1974). Such lesions typically occur in hyperpigmented individuals associated with congenital ocular and dermal melanosis or other congenital dermal changes (skin naevi, naevus of Ota and naevus of Ito); the prognosis is good, but malignant transformation may occur later in life. *In the series of Verdaguer (1965)*

four out of seven malignant choroidal melanomas in young patients (7–20 years old) arose in pre existing ocular melanoses In contrast the usual "adult ma lignant melanomas probably arise *de novo* in sparsely pigmented white people often with blue or light iris colour

As for our own case we should on this background emphasize that

The melanoma of the choroid showed a typical adult pattern

The melanin content was *not* on a specially low infantile level (as suggested by Badtke & Zimmermann (1956) and Burki (1961)) in spite of the very low age of the patient (11 months only)

The malignancy can not be questioned The growth potential was obvious and pleomorphic cell aggregates were seen inside vessels and vascular sinusoids We have however no evidence of metastatic spread

The origin of the tumour was the choroid The iris was intact

The patient was a typical Scandinavian blond type without evidence of pre existing melanosis in the enucleated eye or elsewhere

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The Department of Ophthalmology (Heads I Dreyer J Edmund E Gregersen Sv Vedel Kessing H H Seedorff) and Department of Paediatrics TG (Head E Winge Flensborg) Rigshospitalet University of Copenhagen Denmark

CONJUNCTIVAL GOBLET CELLS IN PATIENTS WITH CYSTIC FIBROSIS

BY

KÅRE HOLM and Sv VEDEL KESSING

In five patients with cystic fibrosis of the pancreas the mucous glandular system of the conjunctiva was studied as changes if any in the conjunctival goblet cells might be applicable as a diagnostic test in questionable cases
A whole mount technique was used specially developed for studying conjunctival goblet cells In all five cases the qualitative as well as quantitative goblet cell findings were in accordance with a previously reported normal material In particular there were no signs of stagnated secretion

Key words cystic fibrosis – conjunctival mucous glandular system – goblet cells – stagnated secretion

Cystic fibrosis (CF) is an inborn error of metabolism whose symptoms are due to dysfunction of the exocrine glands Clinically the disease is characterized by symptoms and signs from the lungs (chronic obstructive lung disease) and alimentary tract (malnutrition and chronic dyspepsia)

Its prevalence in the Copenhagen area during the period 1958–1962 was found

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to be 1:2893 (Lykkegård Nielsen 1973). The diagnosis is based on the clinical findings as well as on increased sodium in sweat tests and decreased trypsin in the duodenal juice.

As a normal sweat test does not rule out CF (Wange Flensborg 1971), a further diagnostic test is needed in such doubtful cases. Tygstrup et al. (1972) recommend biopsy from the lips, as 72% of CF patients have typical histological changes with stagnated secretion in the labial glands.

CF may give rise to ocular signs in the form of retinal changes (cystic degeneration of the macula, venous engorgement, papilloedema. Bruce, Denning & Spalter 1960). As there have been no investigations into the glandular system of the eye in this disease, we undertook qualitative and quantitative studies of the conjunctival goblet cells in five children with CF. The assessment was based upon previous studies on the normal mucous glandular system of the conjunctiva (Kessing 1968).

Material

Five children aged 3–11 years, all with a typical history. Early onset of malnutrition, chronic dyspepsia and chronic obstructive lung disease. All the patients had clearly elevated sweat sodium levels on at least four occasions in the pilocarpine iontophoresis sweat test (Gibson & Cooke 1959) (Fig. 1) and absent or low trypsin in the duodenal fluid. At examination for fibroblast metachromasia (Danes & Flensborg 1971) two proved to belong to class 2 (metachromatic) and two to class 3 (ametachromatic). One patient was not examined for metachromasia (Table I).

Method

A previously described whole mount technique was used (Kessing 1968). Circular conjunctival biopsies, diameter 5 mm, were removed from a site 5–10 mm inferotemporally to the limbus cornea, stained by a modified combined PAS Alcian blue staining and rendered transparent by a special clearing fluid (cf. Kessing 1968).

Without any sectioning it is thereafter possible to perform qualitative and quantitative studies of goblet cells with great accuracy (Kessing 1968).

Table 1
Clinical and laboratory investigations of the material

CF No	Age at diagnosis	Age at biopsy (years)	Malnutrition from	Chronic dyspepsia from	Chronic *obstruct* lung disease from	Sweat Na * iontophoresis sweat test (Gibson & Cooke)	Trypsin activity in duodenal content	Skin fibroblast class (Danes)
35	0 + 9 mths	12	birth	6 months	< 1 month	140–138–176–192	0	3
51	0 + 5 mths	6	2 months	? months	2 months	110– 91– 77– 70	low	3
95	1 + 11 mths	9	? months	? months	1 month	99–104– 77– 85	0	2
10?	0 + ? mths	10	birth	1 month	1 month	88–111–111– 94	0	2
1?8	5 + 5 mths	8	8 months	birth	1 year	103–10?–100– 99	0	?

Normal values Below 60 meq/l

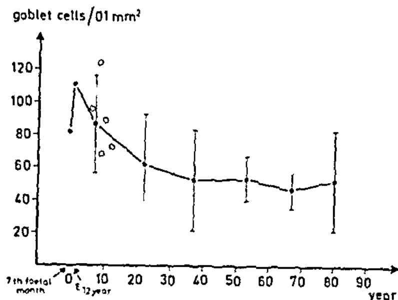


Fig 1
Density of goblet cells in the five patients with cystic fibrosis (○) in relation to the normal material (●) I standard deviation

Results

Qualitatively the goblet cells presented themselves as round bodies diameter 15-20 μ The goblet cell secretion taking PAS as well as Alcian blue stain exhibited the usual thready gel configuration

Quantitatively the mean density of goblet cells in the material was 96/0 1 mm² The findings in the individual specimens are given in Fig 1 in relation to the normal material (Kessing 1968)

Discussion and Conclusion

Stagnation of secretion in the conjunctival goblet cells is a normal age phenomenon occurring with increasing frequency from the age of 20 (Kessing 1969) *Goblet cells with stagnated secretion range in size* from 20 to 40 μ and unlike normal goblet cells they contain predominantly neutral Alcian blue negative mucopolysaccharides in amorphous granular masses (Kessing 1969) None of the

present specimens contained goblet cells with stagnated secretion Normal conjunctival goblet cells have a diameter of 15–20 μ and are PAS as well as Alcian blue positive (Kessing 1968)

Thus it may be concluded that the qualitative goblet cell studies revealed normal findings

In the age group 1–15 years the goblet cell count normally averages 87/0 1 mm (standard error of the mean ± 8) in that conjunctival area in which the CF patients showed an average of 96 goblet cells/0 1 mm It is apparent also from Fig 1 that the goblet cell density for the individual patients was within the normal average ± standard deviation (s d ± 30) in all cases but one

It is concluded therefore that the quantitative goblet cell findings too were normal in patients with CF

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OCULAR CALCIFICATIONS IN PRIMARY HYPERPARATHYROIDISM

Histochemical and ultrastructural study of a case Comparison with ocular calcifications in idiopathic hypercalcaemia of infancy and in renal failure

BY

O A JENSEN

In a case of primary hyperparathyroidism ocular changes were observed as vacuoles in the basal cells of the corneal epithelium and by staining procedures calcium was found in the corneal and conjunctival epithelia the corneal endothelium and in the sclera
By electron microscopy needle like crystals of calcium hydroxyapatite precursors were found intracellularly also in keratocytes The crystals in the epithelial cells were often confined to the nucleus
In a case of idiopathic hypercalcaemia of infancy similar crystals were found intracellularly in the corneal epithelial and stromal cells and in this case extracellular deposits morphologically identical with extra cellular depos ts in conjunctival biopsies from patients with renal failure were also seen These extracellular aggregations were probably also com posed of hydroxyapatite
The difference in morphology between intracellular and extracellular de posits is discussed in the light of the serum values of the mineral ions found in the three groups of cases

Key words ocular calcifications – primary hyperparathyroidism – idio pathic hypercalcaemia of infancy – renal failure – histochemistry ultra structure

Presented in an abbreviated and modified form at the 13th Annual Meeting of the European Ophthalmic Pathology Society Toulouse May 1974

Calcifications in the eye are fairly common particularly in eyes with long standing diseases Corneal calcifications in band shaped keratopathy calcifications of the choroid and retina in phthisical eyes scleral calcifications in senile eyes and calcifications in tumours as retinoblastomas and gliomas are well known In such cases it was considered that *calcium was deposited as simple carbonates and phosphates* However Fine (1969) showed that calcium is deposited as *hydroxy apatite* extracellularly in human band keratopathy Calcium bound as calcium *oxalate* may be found in the retina subretinally and in the pigment epithelium in cases of long standing detachment and in generalized oxalosis from various causes (see Jensen 1974 this issue) In all the above mentioned cases the calcium is deposited independently of the blood calcium

Calcifications in various ocular tissues are also observed in diseases where *serum calcium is elevated and the same holds true in diseases such as renal diseases* where the serum phosphates may be changed

The present report concerns the intracellular deposits of calcium in a case of primary hyperparathyroidism with highly increased serum calcium an electron microscopical study of its morphological pattern and composition and comparison with the calcifications in a case of idiopathic hypercalcaemia of infancy (to be published separately) together with calcifications in conjunctival biopsies from patients with renal failure (increased serum phosphorus) This biopsy *material has previously been published as a clinical and histopathological study* (Ehlers Kruse Hansen Hansen & Jensen 1972)

Material and Methods

Clinical history (Skive sygehus med dept rec no 190070-1757)

A 57 year-old previously healthy woman had for 6 months noticed an increasing sensation of fatigue anorexia loss of weight and pain in the lower back A sudden pain in the left hip with subsequent loss of strength in the leg led to admission to hospital

X ray disclosed an osseous destruction of the left iliac bone and calcification of the kidneys The patient had impaired renal function, and several *blood tests demonstrated* high values of serum Ca (16 mg%) serum alkaline phosphatase and a high blood sedimentation rate (106 mm h) Serum phosphorus (5 5 mg%) was normal

Ophthalmoscopy of the right fundus showed nasally a slightly elevated unsharply delimited grey lesion 3 disk diameters in size Slit lamp examination revealed no corneal or conjunctival changes except for a few lens opacities The vision was unimpaired *Ophthalmologists* considered the lesion to be a metastasis from a primary tumour with osteolytic metastases and secondarily increased serum calcium In spite of much effort no tumour elsewhere was found Two months later the patient was readmitted with a spontaneous fracture of the right collum femoris She died suddenly before therapy was instituted

Pathology (Skive sygehus pat anat inst autopsy no 3??/72-73 Ojenpat inst no 464/73 EM 94)
Autopsy with examination of all organs was performed as well as microscopical examination of heart lungs kidneys spleen, liver thyroid and parathyroid glands adrenals skin and fractured bones

The right eyeball was removed for examination The eyeball was fixed in 10% formalin for 48 hrs It was measured in three dimensions cut and examined in a dissecting microscope and processed according to our routine paraffin technique Deparaffinized hydrated sections were stained as follows

1) Haematoxylin eosin 2) haematoxylin phloxine safranin 3) alizarin red S 4) the von Kossa method 5) murexide staining a m Kaufman & Adams 6) silver nitrate rubeanic acid a m Yasue 7) PAS 8) alcian blue 8 GX Paraffin embedded material was processed for electron microscopy according to a method used by our laboratory (Jensen 1974) One micron epon embedded sections were examined in polarized light and stained with 1% safranin and polychrome stain a m Sato & Shamoto and examined by light microscopy Ultra thin sections were cut with glass knives on a Reichert ultra microtome OM U2 These were examined unstained and after staining with uranyl acetate and lead citrate by conventional transmission electron microscopy and by electron diffraction in a Zeiss EM 9 S 2 electron microscope at 60 KV Paraffin embedded corneal tissue from a case of idiopathic hypercalcaemia of infancy (elfin face syndrome) (Lab no 141/69 EM 119) and conjunctival biopsies of patients with renal failure previously studied light microscopically (Lab nos 712/70 749/70 751/70 EM 111 112 113) were ultrastructurally examined by the same method

Results

Autopsy revealed an adenoma of the right inferior parathyroid gland (diameter 20 mm weight 8 g) calcification of kidneys myocardium splenic vessels and bronchioli as well as osteitis fibrosa cystica with spontaneous fracture of the right collum femoris

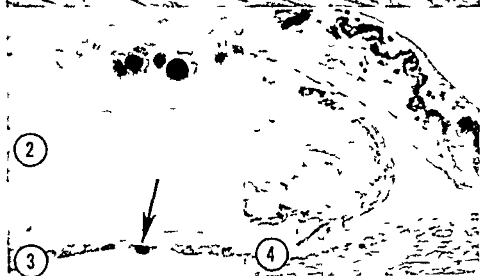
The eyeball was of normal size No corneal or conjunctival changes were observed in the dissecting microscope In the posterior pole a minor lesion a few millimetres in length and half a millimetre thick was seen

Light microscopy

The minor lesion in the posterior pole was found to be a small spindle A melanoma in the choroid

In this study however attention was directed towards the anterior segment In haematoxylin eosin stained sections several cells of the corneal epithelium mainly in the basal layer were vacuolated – even distended – forming small cysts (Fig 1) Bowman s membrane and the remainder of the cornea appeared


1


2
3
4


5

normal In the limbal area and subconjunctivally several basophilic homogene ous masses were noticed in close contact with the epithelium as well as elastosis A positive alcian blue staining was observed here PAS was negative The stain ings for calcium Alizarin red S von Kossa murexide and rubeanic acid were all positive in basal cells of the corneal epithelium (Fig 2) and in a few cells of the more superficial layers A few cells of the corneal endothelium were stained (Fig 3) Several cells in the conjunctival epithelium were likewise stained but a particularly heavy staining was observed in the subepithelial masses in contin uity with the stained epithelial cells (Fig 4) In addition calcium was found in the anterior sclera corresponding to the muscle insertions (Fig 5) Neither Bow man s membrane nor the corneal stroma nor the remaining structures of the eyeball were positive for calcium Epon embedded corneal tissue processed from paraffin embedded tissue showed a faint birefringence in nuclei and cytoplasm

Electron microscopical examination

Needle like crystalline deposits were observed in the cells of the corneal epithe lium in keratocytes and in scleral fibroblasts as well as in epithelial cells of the limbal conjunctiva (Figs 6–9) The heavy masses immediately subepithelially in the conjunctiva could not be studied satisfactorily for technical reasons Cells of the corneal epithelium often contained crystals exclusively in the nucleus (Fig 7) Sometimes the aggregates were to be seen dislodged out of the cell caused by the cutting procedure leaving empty spaces in the cell (Fig 8) The aggregates of crystals varied from 0 2–0 5 μ in diameter (Figs 6 8) and the individual needles had a length of 0 1 μ or less the breadth being about 50 A (Fig 9) The crystals are morphologically identical with experimentally produced precursors of cal

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Fig 1
Vacuolated basal cells of the corneal epithelium Lab no 464/73 Haematoxylin eosin (×275)

Fig 2
Positive von Kossa staining in the basal corneal cells The nuclei are heavily stained. The cytoplasm is granular Note that Bowman s membrane is unstained (×450)

Fig 3
Positive von Kossa staining of an endothelial cell of the cornea (arrow) (×450)

Fig 4
Positive von Kossa staining in the limbal conjunctiva mainly of subepithelial masses (×100)

Fig 5
Positive von Kóssa staining of the anterior sclera (×95)



Fig 6

Star shaped aggregates of needle formed crystals in nucleus and cytoplasm of a kerato cyte C Cytoplasm CT Connective tissue stroma NM Nuclear membrane I M 94 (× 20,500)



Fig 7
Aggregates of crystals exclusively in the nucleus of an epithelial cell of the cornea CB Cell border M Ballooned mitochondrion NM Nuclear membrane (× 11 /00)

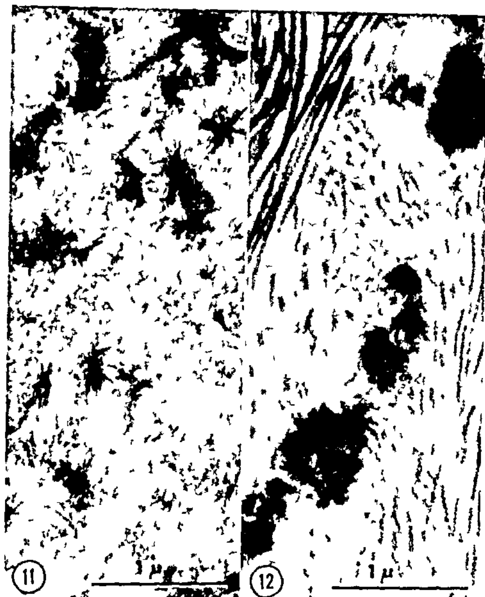


Fig 11

Cell of the corneal epithelium from a case of idiopathic hypercalcaemia of infancy (elfin face syndrome) Note same morphology of crystalline deposits as in the case of hyper parathyroidism NM Nuclear membrane Lab no 141/69 EM 119 (× 34 200)

Fig 12

Extracellular deposits in corneal stroma of same case as Fig 11 The morphology of these aggregates is mainly similar to that seen in renal failure (Fig 10) (× 34 200)

found (Fig 11) In addition extracellular electron dense deposits in the connective tissue stroma were seen In general they appeared similar to the extracellular deposits observed in cases of renal failure but often denser and more homogeneous (Fig 12)

Discussion

The ocular findings can be summarized as follows

A. The case of *hyperparathyroidism* was characterized by vacuolated epithelium of the cornea observed in haematoxylin eosin stained sections (Fig 1) while stainings for calcium revealed this to be deposited in the corneal epithelium and endothelium the conjunctival epithelium and the anterior sclera (Figs 2–5) An overwhelming abundance of calcium in conjunctival cells caused in my opinion the subepithelial masses of the limbal conjunctiva (Fig 4) Ultrastructurally the calcium was observed solely as intracellular deposits of needle like structures in star shaped aggregates in nucleus and cytoplasm (Figs 6–9) In epithelial cells of the cornea the crystals were often confined to the nucleus (Fig 7) The calcium was found to be deposited as hydroxyapatite (Ca_{10} $(Po_4)_6(OH)$) and the crystals had a morphology similar to precursors of the mature crystals which are thicker and faceted

B In the cases of conjunctival biopsies from patients with *renal failure* mainly subepithelial calcifications were observed and only occasionally epithelial deposits In the electron microscope the calcium was seen to be deposited extracellularly as spherical globules with an electron dense periphery and often an electron dense core (Fig 10) Unfortunately it was not possible to find any of the epithelial deposits observed with the light microscope

C In the case of *idiopathic hypercalcaemia of infancy* – a syndrome with facial mental cardiovascular oral and skeletal abnormalities probably an inborn error of metabolism – the calcium was seen ultrastructurally to be deposited intracellularly as precursors of calcium hydroxyapatite crystals often also exclusively confined to the nucleus making the morphology indistinguishable from that of the case of hyperparathyroidism In addition extracellular deposits were observed similar to those in renal failure (Figs 11 12)

The following discussion will mainly concern the difference between intra and extracellular deposits of hydroxyapatite

Berkow et al (1968) published a case of ocular calcifications in a patient with

chronic renal disease hyperphosphataemia and parathyroid hyperplasia Their findings correspond to the findings in the present case of primary hyperpara thyroidism They confirmed by X ray diffraction and electron probe analysis that the deposits were hydroxyapatite They did not describe vacuolation of the corneal epithelium as observed in the present case In this connection findings of Giacomelli et al (1964) are of interest In an experimental study of metastatic renal calcifications by using large doses of vitamin D they found that the tubular cells contained cytoplasmic vacuoles which progressively increased in number and size during the course of their experiment In the vacuoles aggregates of calcium hydroxyapatite crystals were described similar to the aggregates observ ed in the present case of hyperparathyroidism In addition they found extra cellular mineral deposits in aggregates in appearance and size much the same as the extracellular deposits in our cases and similar to aggregations found ultrastructurally in human band shaped keratopathy by Pouliquen et al (1961) Berkow et al (1968) Radnot et al (1970) and in experimental corneal calcifica tion by Fine (1968) and Valouch et al (1974) Berkow et al (1968) succeeded in proving by X ray diffraction that the extracellular deposits in human band keratopathy were composed of hydroxyapatite The extracellular deposits found by Giacomelli et al (1964) in the proximal convoluted tubules of the kidney could not be defined precisely by electron diffraction (as in our cases of renal biopsies) but the diffraction patterns made it not improbable that the deposits were a mixture of apatite and another phase On a purely morphological basis it is then highly possible that the extracellular aggregates in our cases are com posed of hydroxyapatite at least in part Normal as well as abnormal calcifica tion in vertebrates (except for a few types e g calcium oxalate crystals) gener ally implies the presence of hydroxyapatite (Giacomelli et al 1964)

The question then arises why the crystals of hydroxyapatite intra and extra cellularly are different in size and aggregation Since the growth rate of hy droxyapatite crystals is highly dependent upon concentration pH and other physicochemical factors local factors in the cell and in the interstitium may be held responsible for the difference In this respect it is well worth mentioning that the calcifications in our renal biopsies often occurred in areas with elastosis which implies changes in amino acid content (raised glutamic and aspartic acid) Whether it is this change of the acid base equilibrium or as suggested by Sobel et al (1952) the mucopolysaccharides (present especially in this limbal area) which catalytically induce the formation of calcium hydroxyapatite cannot be stated In any case the shape of the crystals intracellularly and extracellularly is different

A study of the present cases of hyperparathyroidism infantile hypercalcaemia and renal failure as well as the clinical data of a number of similar cases from

the literature leads me to suggest that a prerequisite for intracellular deposits is an increased serum calcium and/or some effect on the plasma membrane of the cell probably related to the action of the parathyroid hormone and vitamin D The serum phosphates showed normal levels in our two cases of hyperpara thyroidism (the present case and the one included in our previously published cases of corneo conjunctival changes in renal failure) as well as in four cases published by Porter & Crombie (1973) A raised serum phosphorus thus does not appear to be a prerequisite for the intracellular deposit of calcium hydroxy apatite although phosphates form part of this molecule It is also well known that the parathyroid hormone increases the excretion of phosphates in the urine Secondary renal failure however may at a later stage increase the serum phos phorus in this disease The serum phosphorus was highly increased in our cases of renal failure and in many published cases of idiopathic infantile hypercal caemia (Fay et al 1966 Harris & Ngheim 1968 and Dupont et al 1970) this is probably a factor of significance in the formation of extracellular deposits In addition local physicochemical factors particularly concerning the proteins (as in elastosis and in band shaped keratopathy in both of which the serum ions are normal) probably also play a part

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The National Institute
for the Blind and Partially Sighted
(Head H Skydsgaard)
and The Eye Pathology Institute
(Head S Ry Andersen)
University of Copenhagen Denmark

CALCIUM OXALATE CRYSTALS LOCALIZED IN THE EYE

Subretinal and retinal deposits including deposits in the pigment epithelium

BY

O A JENSEN

Calcium oxalate crystals identified morphologically chemically and histochemically were found in the eyes of two patients one with congenital buphthalmia and one with proliferative diabetic retinopathy The deposits had not been observed clinically or by macroscopic examination during sectioning of the eyeballs Generalized oxalosis could be ruled out as a source of the oxalates The crystals were localized in the detached degenerated retina subretinally and in the retinal pigment epithelium Previously deposition in the last named layer has been reported only once and only in a case of generalized oxalosis The literature is reviewed, and suggestions concerning the formation of localized oxalate crystals are advanced It is concluded that the pigment epithelium is probably involved in the production perhaps through a changed metabolism

Key words ocular calcifications – calcium oxalate crystals – retina – subretinal retinal and pigment epithelium deposits

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The present report concerns the finding of calcium oxalate crystals localized in the eye in two patients without generalized oxalosis. Apart from the sensory retina and especially the subretinal space, crystals were found in the retinal pigment epithelium. This last named localization has recently been found for the first time in a case of generalized oxalosis (*Albert 1973, Bullock et al. 1974*) but never reported in the few earlier reports on calcium oxalate crystals localized exclusively in the eye.

Material and Methods

Case histories

Case 1 (*MB rec no K M 090/47*)
A 26 year old woman with congenital bilateral buphthalmia had her right phthisical eyeball enucleated under pethidine pentothal anaesthesia when she was 9 years old. One *year later a cyclodiathermy of the left eye was performed under trilene anaesthesia* and the procedure was repeated shortly after. At the age of 18 she had a retrociliary diathermy performed under fluothane anaesthesia. The duration of this was 30 min. Vision decreased gradually and huge intercalar staphylomas developed. At the age of 26 her left amaurotic eyeball was enucleated again under fluothane anaesthesia for 30 min. Ophthalmoscopic examination of the fundus had not been possible for the last 4–5 years mainly because of cataract but punctate lesions of the fundus had never been described before that time. No general disease was revealed, particularly no renal disease.

Case 2 (Niels Steensens Hospital rec no 11608, Rigshospitalet dept F rec no N 5 0 66–6, MB rec no F S 1 0891)
A 53 year old woman had diabetes mellitus since the age of 21, the last 11 years with progressive proliferative diabetic retinopathy. Her last affected eye – the right eye – was photocoagulated twice under local anaesthesia. In spite of this the vision deteriorated to light perception and uveitis, consecutive glaucoma, complicating cataract and bullous keratopathy gradually developed. The right eyeball was enucleated under halothane (*fluothane*) anaesthesia of *30* minutes' duration. *Fundus abnormalities apart from* the proliferative diabetic changes had never been noticed. There had been no evidence of diabetic nephropathy.

Histopathology (Ojenpat inst nos 82/57, 1559/73, 24/74)
All three eyeballs were fixed in 4% buffered neutral formaldehyde for 24 hours. The *left eyeball in case 1 and the right eyeball in case 2 were measured in three dimension*, examined in a dissecting microscope and processed routinely according to our paraffin technique. Deparaffinized hydrated sections were stained with haematoxylin eosin, haematoxylin phloxine saffranin, murexide, alizarin red S, the von Kossa method and silver nitrate rubeanic acid a.m. Yasue without exposure and after exposure to acetic acid for 30 min. Furthermore some solubility tests applied to deparaffinized sections were made according to Zimmerman & Johnson (1958). The sections were studied microscopically in transmitted and in polarized light and the deposits measured

with a Zeiss object micrometer Agfa Isopan IF (17 DIN) and Agfacolor CT 18 (18 DIN) without a filter and with a Wratten 80 A filter were used for photographic recording

Results

The left eyeball in case 1 was greatly increased in size (26 x 26 x 26 mm) The diameter of the intransparent cornea was 14 mm the sclera thin and the remaining structures atrophic as is usual in buphthalmic eyes The gliosed retina was totally detached Some proliferation of the pigment epithelium otherwise of normal appearance was present as well as subretinal haemorrhages fresh and old In the retina and particularly subretinally numerous crystals and aggregates of crystals were observed often so close as to form a subretinal layer (Fig 1) The aggregates were star shaped or comb shaped varying much in size from 0 02 mm to 0 1 mm The largest single crystals making up the aggregates were 0 05 x 0 005 mm The crystals were birefringent (Figs 1 B 2 B) An unusual finding was a foreign body reaction although it was slight (Fig 3) By using polarized light fine birefringent crystalline structures were found in the pigment epithelium sometimes half way out of this as if they were under secretion (Fig 4) The von Kossa murexide and alizarin red S stainings were mainly negative but some aggregates showed centrally a positive staining (Fig 5) The silver nitrate rubeanic acid staining was highly positive also after exposure to acetic acid (Fig 6) These staining reactions are indicative of calcium oxalate probably with some admixture of phosphates The silver nitrate rubeanic acid staining after acetic acid treatment can in particular be considered as reasonably selective for calcium oxalate (Chaplin 1974) The crystals were found to be insoluble in water absolute aethanol xylene and 6 % acetic acid for 24 hours but soluble in 1 N HCl for 20 min (Fig 7) and in 0.5 % periodic acid for 3 hours The chemical properties are likewise indicative of calcium oxalate

It was possible to re examine the right eyeball in case 1 enucleated 17 years earlier It was found to be phthisical with ossifications of the choroid and proliferation of the pigment epithelium The gliosed retina was totally detached No crystalline deposits were found

The enucleated right eyeball in case 2 was of normal size Pronounced diabetic changes were found in the iris and retina In the sensory retina especially subretinally and in the retinal pigment epithelium similar crystalline aggregates as in case 1 were found showing precisely the same birefringence chemical and staining properties as the deposits in case 1 In none of the eyeballs did macroscopic examination during sectioning reveal a flecked retina



Fig 1 A

Subretinal layer [illegible] aggregates of crystals Lab no 74/74 Haematoxylin eosin ([illegible])

Fig 1 B

Same area in polarized light The birefringence of the aggregates is depicted as white areas

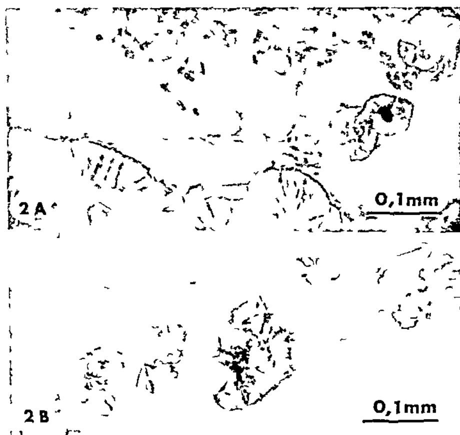


Fig 2A
Round and comb shaped aggregates of crystals Lab no 1589/73 Haematoxylin eosin (× 275)
Fig 2B
Round aggregates in polarized light showing star and mulberry formation Lab no 1589/73 Haematoxylin eosin (× 27)

Discussion

Earlier cases of calcium oxalate crystals localized in the eye have been published by Flocks et al (1955) They found lenticular deposition in 18 % of 138 cases of glaucoma associated with hypermature cataract. The crystals were lying in the sclerotic nuclei of the Morgagnian cataracts

Retinal deposits were observed by Cogan et al (1958) Zimmerman & Johnson



Fig 3
Foreign body giant cell in degenerated retina with crystals in cytoplasm (arrows) Lab no 2474 Haematoxylin eosin (x 275)

Fig 4
Birefringent crystals and crystal aggregates in pigment epithelium Figs 4 A and B – Lab no 24 /4 Figs 4 C, D and E – Lab no 1559 /3 Haematoxylin eosin Polarized light (x 450)

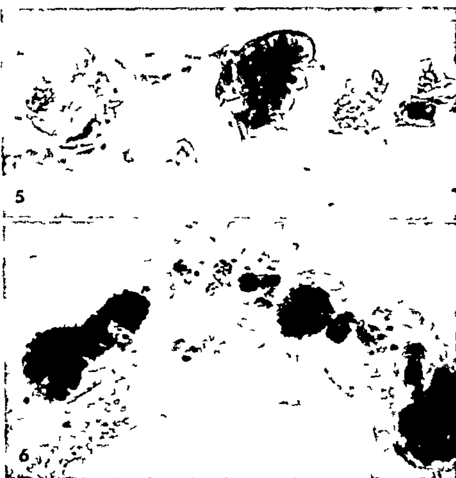


Fig 5
Positive von Kossa staining in core of crystal aggregates Several negatively stained minor aggregates are seen Lab no 1589/73 (x 275)

Fig 6
Highly positive silver nitrate rubeanic acid staining a m Yasue after exposure to 2 M acetic acid for 30 min Lab no 1589/ 3 (x 275)

(1958) Garner (1973) and Friedman & Charles (1974) In all these cases the crystals were lying as in the present two cases subretinally and in the outermost layers of the degenerated retina They were in most cases associated with old detachments often in traumatized eyes the cases of Friedman & Charles being in the retina of diabetic patients Crystals in the retinal pigment epithelium were not described in any of these cases

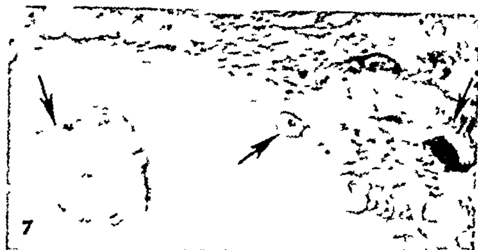


Fig. 7
Total and partial dissolution of crystals (arrows) after exposure to 1 N hydrochloric acid for [illegible]0 min Lab no 155[illegible] 3 Haematoxylin eosin ([illegible])

Although calcium oxalate deposition in various organs especially the kidneys is well known in cases of generalized oxalosis the first ocular case in generalized oxalosis was described recently (Albert 1973 Bullock et al 1974) They found the crystals in the retina but also for the first time in the pigment epithelium as seen light microscopically and ultrastructurally These writers were able to associate the histopathological findings with the clinical picture of the flecked retina syndrome of Krill & Klien (1965) a fundus picture with presumably several aetiologies of which oxalate crystal deposition may be only one Furthermore these writers demonstrated a connection with methoxyflurane anaesthesia This anaesthetic is nephrotoxic and according to Mazze et al (1971) inorganic fluoride and oxalic acid result from the biotransformation of methoxyflurane Mazze et al found no significant elevation of these products in serum and *urine in patients anaesthetized with halothane (fluothane) The patient of Albert (1973) and Bullock et al (1974) had been exposed to prolonged methoxyflurane* anaesthesia and widespread oxalosis was found at autopsy

In the present two cases the morphological appearance of the crystalline deposits as well as their chemical and staining properties were wholly identical with all the above mentioned cases identifying the deposits in the present cases as calcium oxalate crystals The most exact test i e X ray diffraction applied in some previous cases was not carried out in the present cases In this respect it is interesting that Cogan et al (1958) in accordance with the histochemical

findings of the present cases found by X ray diffraction relatively small amounts of calcium phosphate mixed with the calcium oxalate

Regarding the aetiology the present two patients had been exposed to short fluothane (halothane) anaesthesia only and had no general symptoms particularly no indication of impaired renal function and they are still in good general condition As mentioned above fluothane is not nephrotoxic and probably not hepatotoxic as previously suggested (Schumer et al 1971 Almersjo 1972) Thus a connection with the anaesthesia can be ruled out in the present cases It is interesting that crystals were found in the retinal pigment epithelium in the present cases of localized calcium oxalate deposition as in the above mentioned case of generalized oxalosis In cases of generalized oxalosis the origin of the crystals is evident but not in the localized cases Oxalates are not produced in normal endogenous metabolism Oxalate crystals have been produced experimentally in the vitreous body retina and lens of rabbits by feeding them naphthalene (Pirie 1968) Metabolic products (1 2 naphthaquinone) from the breakdown of naphthalene oxidize ascorbate followed by breakdown of this to oxalate In these experiments the pigment epithelium was also damaged but it was not possible to decide whether the changes of the pigment epithelium occurred before those in the retina proper

Garner (1973) suggested that the intra ocular ascorbic acid could be the source from which oxalate crystals were formed and also suggested that phenylalanine and/or tyrosine from the pigment epithelium could perhaps be the source He also pointed out that the mode of crystal growth suggests a seeding of calcium oxalate contained in the subretinal exudate

The finding of crystals in the retinal pigment epithelium in the case of Albert (1973) and Bullock et al (1974) in the present two cases and in the experimental findings of Pirie (1968) makes it quite probable that the pigment epithelium is involved in the production This might be due to some block in the citric acid cycle i e between oxalic acid and pyruvic acid or succinic acid and fumaric acid or by a changed phenylalanine tyrosine metabolism Quinones are oxidizers and dopaquinone – an intermediate in the formation of melanin from tyrosine – might well be the oxidizer of ascorbic acid as naphthaquinone was in the experiments of Pirie (1968)

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OBSERVATION OF KOLMER S CRYSTALLOID OUTSIDE THE RETINA

Presence in the Corneal Endothelium in Various Conditions

BY

O A JENSEN M J HOGAN and IRMGARD WOOD

A structure similar to Kolmer s crystalloid an organelle found in the horizontal cells of the retina was observed in endothelial cells of the cornea from four patients (one case of hyperparathyroidism and three cases of corneal dystrophy) Based on a morphological analysis it is concluded that the structures in the present cases are identical with the cytoplasmic structure of the horizontal cell and that the membranous tubes probably constitute a special form of rough surfaced endoplasmic reticulum eventually involved in transport and/or a specialized protein synthesis

Key words Kolmer s crystalloid – extraretinal localization – corneal endothelium – ultrastructural observation

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In 1918 Kolmer described a basophilic crystalloid structure in the cytoplasm of the horizontal cells of the retina.

Ultrastructural examination has revealed this cytoplasmic organelle to be composed of tubular elements with ribosomes on the membranous inner walls of the tubes (Fig. 1).

The function of this organelle is unknown, but it is considered to be a morphological characteristic of the horizontal cell, helping in identifying it. It has never been described in the eye outside the retina.

This report concerns the finding of cytoplasmic structures similar to Kolmer's organelle in endothelial cells of the cornea in various lesions.

Material and Methods

The four corneas in which the observations were made came from a) an eyeball enucleated post mortem from a patient with hyperparathyroidism and b) from three patients with various corneal dystrophies.

a) The eyeball (Øjenpat. inst. no. 464/73, EM 94) was obtained at autopsy from a patient having an adenoma of the right inferior parathyroid gland. It was fixed in 10 % formalin and processed routinely. Paraffin embedded material later was prepared for electron microscopy using a technique previously described from our laboratory (Jensen 1974). Ultrathin sections of epon embedded material were cut with glass knives on a Reichert ultramicrotome OM U2 and examined in a Zeiss EM 9 S 2 electron microscope unstained as well as after staining with 1 % uranyl acetate for 45 min and 1 % lead citrate for 5 min.

b) *The three dystrophic corneas obtained as buttons during corneal transplantation* came from a case of Fuchs dystrophy (Eye Pathology Lab. San Francisco no. EM 1107), a case of Groenouw I (nodular dystrophy) (Eye Pathology Lab. San Francisco no. EM 1235) and a case of Chandler's syndrome (Chandler 1956) (Eye Pathology Lab. San Francisco no. EM 1402).

Each corneal tissue was fixed with 3 % paraformaldehyde-glutaraldehyde at pH 7.3 in 0.2 M Na cacodylate buffer with the addition of 1 % sucrose, 0.2 % calcium chloride and 1 ml of 0.73 M potassium chloride for 2 hours at room temperature.

The specimens were then washed in a solution of one part 0.2 M Na cacodylate, one part 0.5 M sucrose and post fixed in 2 % OsO_4 in Na veronal acetate buffer with the addition of sucrose at 4°C. After 2 to 3 hours of post fixation the tissue was washed in 0.05 M Na H maleate buffer pH 5.2 and stained *en bloc* with 2 % uranyl acetate in Na H maleate buffer pH 6.0 for 9 hours (dark), dehydrated in graded acetone 70–100 % and embedded in araldite.

Ultrathin sections were stained with 2 % uranyl acetate for 45 min and lead citrate for 15 min and examined in a Siemens Elmiskop I.

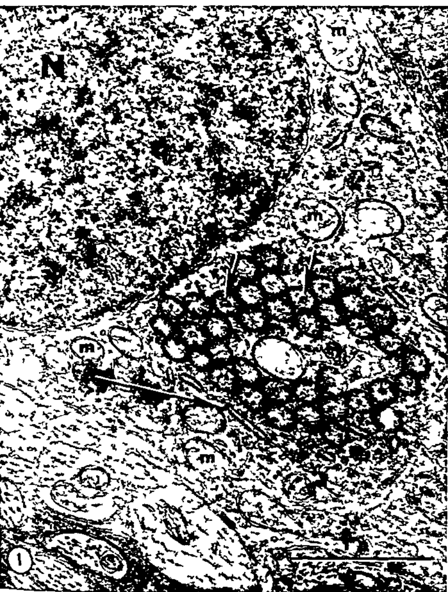
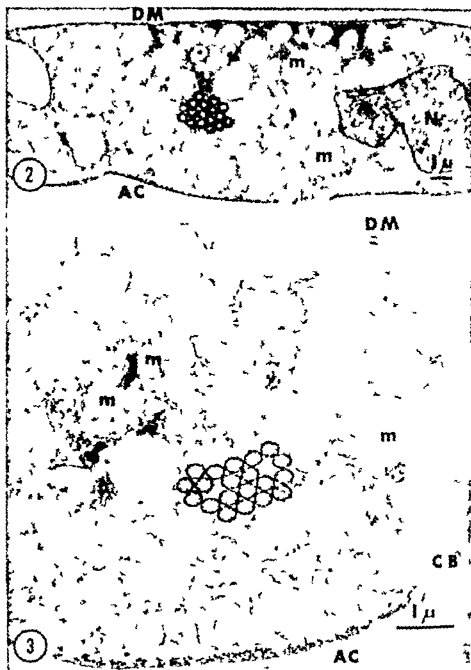


Fig 1

Kolmer's crystalloid in the cytoplasm of a horizontal cell of the retina Note rough surfaced endoplasmic reticulum (long arrow) and central tubules (short arrows) m = mitochondria. N = nucleus Compare with Fig 9–53 p 461 in Hogan Alvarado Weddell *Histology of the Human eye* (see references) (× 36 000)



Figs 2 and 3

Results

a) The corneal endothelium of the paraffin embedded eyeball had the usual appearance of autopsy paraffin embedded tissue when it is subsequently pro cessed for electron microscopy i e the main structures were rather well preserved in contrast to the cytoplasmic organelles

The nucleus and the plasma membrane were rather distinct but the mito chondria were ballooned (Figs 2 3) Endoplasmic reticulum in the usual form could not be identified In several cells (Figs 2 3) however a well preserved structure similar to Kolmer s crystalloid of the retinal horizontal cell (Fig 1) could be seen It was composed of 19–20 uniform tubes forming a lattice like pattern The whole structure was approximately 1 x 2 μ The single oval tubes had an outer and inner membrane the outer diameters being 0 25 x 0 3 μ and the inner 0 2 x 0 25 μ (Fig 4) Ribosomes were numerous near the membranes partly lying between the two membranes and partly along the inner one Direct attachment of ribosomes to membranes could not be made out in the rather fuzzy pictures Remnants of possible central tubules could be seen in a few tubes (Fig 4) similar to the central tubules found in Kolmer s crystalloid of the hori zontal cell (Fig 1)

b) In the optimally fixed tissue from the three corneas with dystrophies (Figs 5–9) similar tubular structures were found However the number of tubes was lower (varying from two to seven) and they were not so closely arranged making the lattice like pattern less conspicuous The membranes of the tubes were more distinct and better separated and the outer diameters were about 0 5–0 6 μ the inner being about 0 4 μ

In some of these tubes a third central tubule was also observed (Figs 6 7 8) often with an oval cross section the diameter being about 0 1–0 15 μ (Figs 7 8) This tubule could be a shorter membranous sac and therefore might not be revealed in all tubes cut in a single plane Ribosomes were absent in relation to these central tubules A very significant finding was the presence of rough surfaced endoplasmic reticulum (RER) in relation to the tubular structures (Figs 5 7)

Fig 2

Crystalloid in an endothelial cell of the human cornea in a case of hyperparathyroid ism. Note the ballooned mitochondria due to previous paraffin embedding technique AC = anterior chamber DM = Descemet s membrane m = mitochondria N – nucleus Øjenpat inst no EM 94 (x 5,500)

Fig 3

Crystalloid in another endothelial cell of the same case as Fig 2 Abbreviations as in Fig 2 CB = cell border Øjenpat. inst no EM 94 (x 14 600)

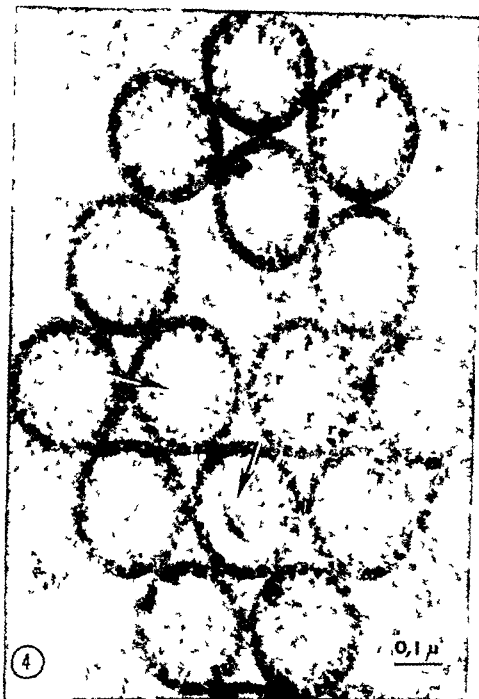


Fig 4

The tubes are probably composed of a double membrane Ribosomes (r) are abundant on the inside of the membranes In one tube a central tubule is seen and the pr[illegible]ble remnants are found in another one (arrows) Öjenpat inst no EM 94 ([illegible]

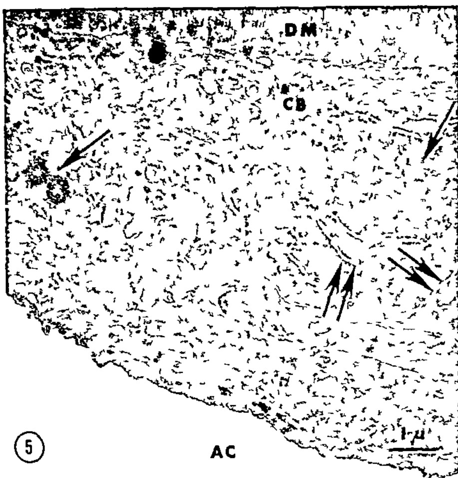
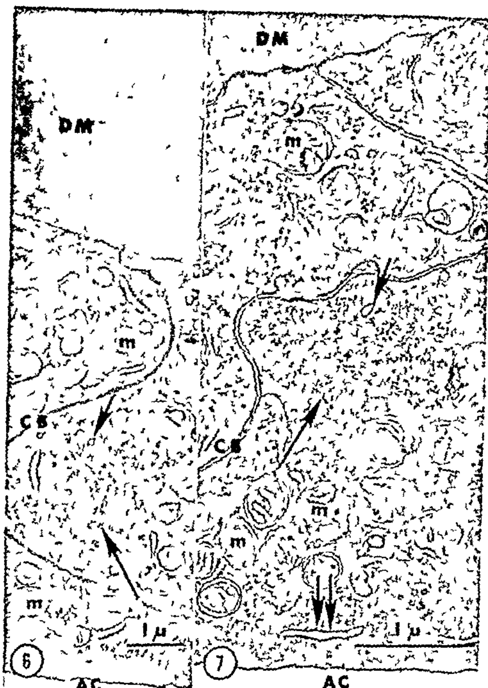


Fig 5

Tubes forming a crystalloid pattern (arrows) in the endothelium of the human cornea in a case of Fuchs dystrophy Note the normal looking rough surfaced endoplasmic reticulum (double arrows) Abbreviations as Figs 2 3 Eye Pathology Lab San Francisco no EM 1107 (× 12 000)

Discussion

Kolmer found the crystalloid of the horizontal cell mainly in the peripheral parts of the human retina and only in adults (Kolmer 1918) He subsequently examined the retinas of various animals but found the structure only in that of the chimpanzee (Kolmer 1936) He realized that the structure was different from the



Figs 6 and 7

System of tubes (long arrows) in corneal endothelium in a case of nodular dystrophy (*Groenouw I*) Note a central tubule without ribosomes (small arrows) (see also Fig 8) Normal looking endoplasmic reticulum present (double arrow) Abbreviations as Figs 2 3 Eye Pathology Lab San Francisco no EM 1938 (x 13 500 x 30 000)

crystal of Reinke found in the interstitial cells of the human testis This was later confirmed by ultrastructural studies He further suggested the crystalloid to be composed of proteinaceous material

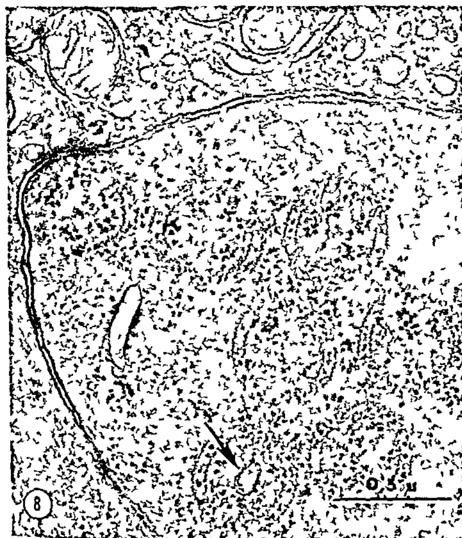


Fig 8

System of tubes in another cell of the same case as in Figs 6 7 Two membranes are clearly seen with numerous ribosomes (r) in the neighbourhood In one tube a central tubule or sac without ribosomes is noted (arrow) Eye Pathology Lab San Francisco no EM 1938 (× 60 000)

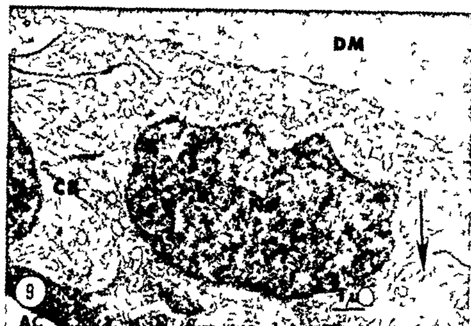


Fig 9
Tubes (arrow) in a corneal endothelial cell in a case of Chandler's syndrome Abbreviations as in Figs 2 3 Eye Pathology Lab San Francisco no EM 1402 (x 500)

Ultrastructural examinations of retinal horizontal cells during the last 10 years have revealed a cytoplasmic tubular structure without a crystalline structure (Missotten 1961 1964 Yoshida 1965 Yamada 1966 Uga & Ikui 1969 Hogan Alvarado & Weddell 1971 Fine & Yanoff 1972)

Uga & Ikui (1969) stated that the morphology of the wall of the crystalloid body differed from that of usual cell membranes and they maintained the view of a crystalline lattice as described in oocytes (Ward 1962) and phagocytes (Karasaki 1965) A study of the illustrations in the publications of these authors in our opinion shows a completely different structure i e intranuclear and intramitochondrial crystalline often hexagonal and much more like the crystal of Reinke However the tubes in Kolmer's crystalloid are arranged so close in a lattice like pattern that they may give the impression of a crystal structure

The description by the various authors is concurrent and the morphology of the organelle is as depicted in Fig 1

Structurally it is a stack of tubular elements about 40 in number (but up to 63 have been observed (Uga & Ikui 1969)) each with a diameter of 0 2-0 25 μ and several micra in length The ends are considered to be open Each tube

consists of two membranes a double unit membrane with numerous ribosomes along the membrane In some tubes saccular structures without ribosomes may be found centrally

Comparing Fig 1 with Figs 2 3 and 4 the very close resemblance in the composition and the arrangement of the tubular structures is evident Only the number is different

The number of tubes in Figs 5–9 is even less the distance between the two circular membranes is larger and the arrangement of the single tubes more irregular The overall pattern in these cases is apart from the number of tubes more like the illustration of Kolmer s crystalloid by Uga & Ikui (1969) We ascribe this difference to more or less good fixation and/or preparation which is obvious in our case of hyperparathyroidism where previously paraffin em bedded autopsy tissue was used

Particularly in this case it could be postulated that the whole structure is an artifact eventually representing a shrunken rough surfaced endoplasmic reticu lum (RER) Against this argument is the close resemblance between the struc ture in Fig 1 (Kolmer s crystalloid in a horizontal cell) and that in Figs 2 3 and 4 Also because RER of normal appearance is present in the horizontal cell in addition to the characteristic Kolmer s crystalloid (Fig 1) In our optimally fixed corneas a normal looking RER is also present together with the tubular structures

In our opinion the lattice like tubular structure in the present cases is identical to that found in the horizontal cell of the retina We consider it a special form of RER and although it is well known that RER may assume a variety of forms as a reaction to injury (Bulger Griffith & Trump 1966) it may also vary depend ing on the stage of development and activity (Behnke & Moe 1964) We accept the structure as real

Ribosomes may arrange themselves in crystalline arrays in special cells (Bar tels & Weier 1967 Taddei 1968) but so far the special arrangement adjacent to tubular membranes forming a lattice like structure has been reported outside the horizontal cells of the retina only in renal proximal tubular cells of three species of primates (Bulger 1968) in the vascular endothelium of the frog (Stehbens 1965) and now in corneal endothelium

The function of this special form of RER is unknown but hypothetically one could assume an association with transport mechanisms considering the cells in which it has been observed Another possibility is a site of a specialized protein synthesis particularly regarding the endothelial cells of the cornea which are involved in corneal nutrition and in the synthesis of Descemet s membrane So far however the crystalloid structure has only been observed in pathological corneas

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LYMPHOMA AND OTHER LYMPHOID LESIONS OF THE ORBIT

Preliminary Report

BY

JORGEN KLEENER

The orbit differs from the rest of the organism excluding the central nervous system as concerns lymph drainage This may possible explain some of the peculiar features in lymphoid orbital lesions
The lymphoid tumours of the orbit are discussed on the basis of the classification most widely applied An illustrative case is reported and it is concluded that even if local therapy may prove successful patients in whom orbital lymphoid tumours have been diagnosed should be kept under constant observation with a view to prompt institution of treatment upon evidence of generalized disease

Key words orbit – tumours – orbital lymphoma – malignant lymphoma – reactive lymphoid hyperplasia – lymphoproliferative disease

Orbital neoplasms are tumours situated in the orbit behind the orbital septum but outside the globe The most frequent manifestation of an orbital tumour is exophthalmos Double vision and diminution of vision are also common features (Jackson Coleman et al 1972 Adam & Farr 1971 and Godtfredsen 1947)

There are malignant and non malignant growths that are morphologically similar to those found elsewhere in the body The lymphoid tumours are how

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ever of special interest since the orbit differs from the rest of the organism excluding the central nervous system in that the existence of lymph nodes or of functioning lymphatic vessels has never been established (Hogan & Zimmerman 1962 and Ingalls 1953) Though many investigators have maintained that lymph vessels are non existent lymphangiomas seemingly do occur in the orbit (Boniuk 1964 and Hogan & Zimmerman 1962)

Tumours situated anterior to the orbital septum may metastasize to the pre auricular glands or to the cervical lymph nodes This is not seen with tumours of the orbit unless the conjunctiva or the eyelids are involved

In the past lymphoid tumours and tumour like lesions were classified as benign inflammatory lymphoid (reactive) pseudotumours and malignant in flammatory neoplasms (Adam & Farr 1971 Godtfredsen 1947 and Stark & Thiel 1970) They have been the subject of continuous dispute and uncertainty clinic ally as well as pathologically which is reflected in the classification generally used today (Boniuk 1964)

The inflammatory tumour like lesions (sclerosing inflammatory lesion chronic myositis nodular fasciitis lipogranuloma and other granulomas etc.) are outside the scope of this paper

Lymphoid tumours and tumour like lesions may be divided into three broad categories

1) malignant lymphoma and leukaemia

2) reactive lymphoid hyperplasia (formerly called lymphoid pseudotumour or benign lymphoma)

3) lympho proliferative diseases

Differentiation between these groups is often very difficult A thorough clinical radiological and haematological investigation is always called for (Jackson Cole man et al 1972)

Obvious malignant growths include reticulosarcoma lymphosarcoma Hodg kin s disease and leukaemia Malignant lymphomas consist of lymphocytic cells ranging from well differentiated to highly pleomorphic malignant cells The admixture of other cells is uncommon (Fig 2) except in some types of Hodgkin s disease

Reactive lymphoid hyperplasia is characterized by a heterogenous mixture of variegated cell types such as lymphocytes neutrophilic and eosinophilic gra nulocytes plasma cells reticulum cells and histiocytes Another finding is lymph oid follicles consisting of a central area of germinal centres with a peripheral zone of lymphocytes All cells are well differentiated and show no sign of ma lignancy (Fig 1)



Fig 1

(Eye Path Inst 5041) Orbital lesion of 3 months standing in a 4? year old woman Histologically connective tissue and fat with a variegated mixture of lymphocytes neutrophilic and eosinophilic granulocytes plasma cells and histiocytes are seen Benign ant picture classified as reactive lymphoid hyperplasia ×2 5

Between these two groups – the obviously malignant and the reactive lymphoid hyperplastic – there is a third group the so called lymphoproliferative diseases or disorders characterized predominantly or solely by lymphocytic and reticulum cell proliferation (Fig 1)

This group has not the cytological changes found in the obviously malignant lymphomas and it has not the cellular variegation seen in reactive lymphoid hyperplasia Histologically they are characterized by a mixture of mature lymphocytes and reticulum cells and frequently PAS positive intranuclear bodies (Dutcher Fahey bodies) as in Waldenstrom s macroglobulinaemia are found (Dutcher & Fahey 1959)

In many instances however it is not possible for the pathologist to distinguish with certainty between these groups

Long period observation with repeated clinical examination will eventually reveal the existence of a generalized disease and in some cases the orbital growth is its first manifestation

It is worth noting that even in generalized obviously malignant lymphomas the lymphoid infiltration of the orbit may persist for several years as a reactive hyperplasia Only careful study can reveal whether it is justified to speak of primary as well as secondary lympoma of the orbit

In the past the treatment of the lymphoproliferative types as well as of the reactive lymphoid hyperplasias was limited to surgery and radiation therapy Following surgical biopsy and histological diagnosis radiation has had good and apparently lasting local effect Sometimes quite high doses approaching those used for primary malignant lymphomas are however necessary If in the absence of generalized disease primary malignant orbital lymphoma is suspected radiation therapy of the orbit in tumour doses (approximately 3000 rad for malignant lymphomas) must be considered as indicated by our present knowledge

The following case history illustrates the often unexpected course of these diseases

Case history

A 55 year old woman was admitted to the medical department Frederiksborg amts Centralsygehus Hillerød (FAC) because of back pains The diagnosis was discopathy and she was treated by extension exercises During the admission it was revealed that at age 32 she had been found to have syphilis which had presumably been treated with bismuth Her Wassermann reaction was now 2–3 and she was given 600 000 units of penicillin daily for 10 days WR in the spinal fluid was negative

Seven years later she was admitted to the Eye Department of the University Hospital of Copenhagen (Rigshospitalet Blegdamsvej) with a diagnosis of tumour of the left lacrimal gland She had noticed a small growing mass in her left upper lid Examination revealed a freely movable smooth indolent tumour the size of a walnut in the region of the left lacrimal gland

The tumour and the lacrimal gland (because of involvation) were removed subtotally under local anaesthesia The histological diagnosis (Eye Pathology Institute, no 440 66 Fig 9) was lacrimal gland with lymphoid infiltration ? immune reaction, ? lymphosarcoma or lymphatic leukaemia

Neither the Eye Department nor the Eye Pathology Institute had information of the history of syphilis and the internist consultant concluded No evidence of more generalized disease in the lymphatic system In the case of lymphosarcoma transition – particularly terminally – to lymphatic leukaemia is occasionally seen No indication is found for further medical examination, let alone medical treatment After this the patient was discharged.

Five years later (aged 67) the patient had an increased sedimentation rate (86 mm/h) and increased alkalic phosphatases her Wassermann reaction was 91 TPI was negative In spite of non radical extirpation 5 years earlier there was no recurrence in the orbit

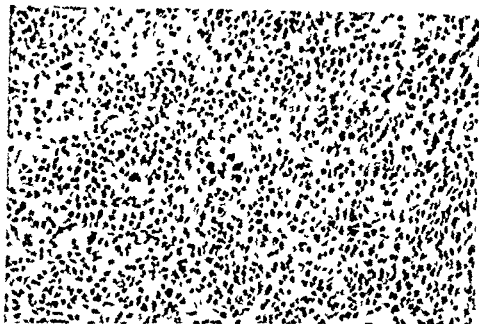


Fig 3
(Eye Path Inst 41/66) Orbital lesion consisting of predominantly uniform lymphoid cells with occasional reticulum cells without mitoses x 275

A large hard growth was found in her left iliac fossa She was referred to the surgical ward and underwent explorative laparotomy which revealed a retroperitoneal tumour the size of a pumpkin

Histological diagnosis of a biopsy (FAC 853/61) (Fig 3) was reticulosarcoma (signed G Hjorth)

Six months later she was again admitted to the medical department (FAC Hillerød) because of a lymph node the size of a chicken egg in her right axilla She was transferred to the Radium Centre (Finseninstituttet Copenhagen) where investigation revealed severely increased WR negative TPI The increased WR was ascribed to the patient's reticulosarcoma

Ophthalmologic examination showed no abnormalities She was treated by cytostatics which she tolerated well and lymphography hereafter showed decreasing lymphomas retroperitoneally and in the pelvis

The tumour responded well to combination therapy (cytostatics prednisone and local radiation therapy) for one year Following a fall in her home her condition deteriorated gradually ending in death after 1 month

Autopsy (FAC Institute for Pathological Anatomy) showed a malignant mesenchymal tumour presumably reticulosarcoma retroperitoneally No orbital or eye changes were observed

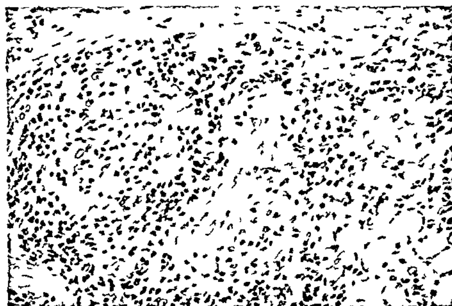


Fig 3

(Eye Path Inst 551/71) Section from retroperitoneal tumour showing a malignant lymphoma with pleomorphous lymphoid cells with several mitoses ×275

Conclusion

To the best of our present knowledge it appears justifiable to stress that once lymphoid infiltration of the orbit has been diagnosed even though local therapy has proven successful patients should be watched carefully for 10 to 20 years with a view to prompt institution of generalized treatment if systematized disease is revealed

The above history suggests that the orbital lesion may possibly represent an immunologic reaction to a generalized malignant disease rather than being a manifestation of this condition

Acknowledgement

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Dedicated to Sigurd Ry Andersen
on the Occasion of his Sixtieth Birthday March 22 1975

NORRIE S DISEASE

Differential diagnosis and treatment

BY

METTE WARBURG

A report on six children with Norrie s disease is presented It is shown that the diagnosis can be established in sporadic cases if the early retrolental opacities are seen and dementia or psychosis occur after a normal infancy Hearing loss is an important diagnostic sign Children with Norrie s disease are more sensitive to disruption of family relationship than most other congenitally blind children

Key words Norrie s disease – blindness – X linked inheritance – mental retardation – infantile psychosis – hearing loss – retrolental opacities – epilepsia

Norrie s disease is a sex linked recessive disorder in which retinal degeneration occurs before or very shortly after birth In one third of the affected individuals hearing loss develops and dementia may set in at any time after the first few years of life EEG dysrythmias have been observed in some of the demented patients and major epileptic seizures have been reported in a few families (Warburg 1965 1966) The diagnosis has hitherto been confined to cases with genetic evidence of the disease The present knowledge of the disease however is sufficient to establish the diagnosis in sporadic cases

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Case Reports

Case 1

P S H b 13 IX 50 This boy was born after an uncomplicated pregnancy 14 days before term No consanguinity or cases of blindness or metal retardation were known in the family An older sister and a younger brother were unaffected The patient received no oxygen treatment He became blind at the age of 5 to 7 weeks Early development was normal At the birth of his younger brother he was sent to a boarding school for the blind at the age of 5 years He attended normal classes until he was 12 years old at which time he was transferred to a home for slow learners where his hearing loss was noticed Behavioural problems were apparent from the age of 11 and aggravated with increasing age He demanded constant attention by adults was disturbing and restless anxious and insecure Intellectually he was considered as a borderline case between low normal and mildly retarded He was given psychotherapy from the age of 15 to 19 years His main problems were anxiety and a fear of giving up his intense dependence on teachers and the psychotherapist Throughout the years of psychiatric treatment he was severely troubled by his love anger for his parents When he was 17–18 years old his performance at school declined music lessons were stopped because of increasing hearing loss he could no longer follow the instruction in arithmetic he experienced depressions and had suicidal thoughts At the age of 21 he was dependent difficult unsatisfiable and of a changing mood his only close friend was an antisocial alcoholic man He could not adjust himself to any working condition and even in a sheltered workshop he could work only under continuous supervision Rehabilitation proved impossible and he was transferred from the rehabilitation centre of the National Institute for the Blind to a home for young persons with a chronic mental handicap He never learned to walk unaided by others out of doors but he could read Braille and type at a low normal speed

Eyes

His mother noticed a white reflex in the pupil when the boy was 3 weeks old At the age of 7 weeks a subtotal detachment with superficial haemorrhages was seen in the right eye while there were grey masses behind the pupil in the left This eye was enucleated on suspicion of retinoblastoma During the first year of life the anterior chamber of the right eye became flat the iris atrophied and the lens became opaque When he was 7 years old a central corneal opacity appeared and when 8 years old a band shaped opacity was seen over the cornea Broad anterior synechiae were then noted and at the age of 16 the eye



Fig 1
Case 1 The histological section shows a normal cornea and iris The lens is cataractous There are elongated ciliary processes adherent to a fibrous vitreous

presented an adherent leucoma The eye eventually became phthisic and deep set in the orbit

Histo pathological examination (Eye Pathology Institute 185/50) showed a normal anterior part There was a falciform retinal detachment with fresh and old haemorrhages and small cystic spaces The vitreous contained proliferating fibroblasts and haemorrhages The ciliary processes were elongated and attached to the vitreous The hyaloid artery was present (Fig 1)

Comment

The patient was unaffected at birth bilateral retinal detachment was noticed at the age of 7 weeks and a histological examination showed a falciform detachment with haemorrhage and elongated ciliary processes adherent to a fibrous haemorrhagic vitreous He was reared in a boarding school from the age of 5 years and at the age of 1? when his hearing loss was evident he was disturbing

dependent anxious and withdrawn Intelligence was probably within the low normal range He thus gradually presented the typical triade of Norrie s disease viz Bilateral retinal non attachment reduced hearing and psychotic behaviour on the basis of which the diagnosis could finally be established

Case 2

P W J b 18 IV 64 This boy was the first child of a secundi gravida born in Germany The first pregnancy terminated with a spontaneous abortion in the second month A hydatiform mole developed in the third pregnancy and the fourth pregnancy was terminated by abortion after genetic counselling The second pregnancy was complicated by bleedings in the sixth week and in the third month The latter was treated with bedrest Librium® (chlordiazepoxide) and Enavid® (norethynodrene and mestanole) There were uterine contractions throughout the rest of the pregnancy Lewis antibodies were demonstrated in the mother s serum at birth Blindness was noted on the fourth day of life Apart from this development was normal during the first 2 years of life He stood at 12 months and began talking at 24 months Then his development stopped his emotional contact with his mother appeared weak and he stopped talking but had good spatial orientation in familiar surroundings At 3 and 4 years of age he had short attacks of high fever without apparent cause During these he cried was remote and presented automatic movements EEG showed dysrythmia and the attacks subsided on antiepileptic treatment At 7 years of age he was severely retarded He understood a few words and was toilet trained Hearing was considered normal Several neurologic examinations revealed nothing abnormal apart from hypermobile joints low muscle tone and a small head circumference (50 cm at age seven) There was increased lanugo hairiness on his body

Eyes

On the fourth day of life bilateral retrolental masses were noted there was no *perception of light and bilateral retinoblastoma was suspected* At the age of 6 days ophthalmological examination showed

Right eye The corneal diameter was 8 mm the cornea was clear the anterior chamber flat and the iris greenish with large vessels There was a persistent pupillary membrane with blood filled vessels and a flat ectropion uveae The lens was clear and the ciliary processes were elongated Behind the lens there was a yellow vascular tissue with superficial haemorrhages In the periphery



Fig 2
Case 9 Right eye at age 11 days Total detachment with superficial vessels Dilated pupil (mydriasis) with ectropion uveae Elongated ciliary processes are seen from 11 to 1 o clock

of the retina a grey detachment was seen (Fig 2) At 7 months of age this eye had shrunken the cornea was opaque and the tension increased When the boy was 13 months old the right eye was deeply set the cornea opaque the anterior chamber flat and posterior synechiae present The chamber angle was closed (Fig 3)

Left eye At 6 days of age the cornea was clear 12 mm in diameter The anterior chamber was of normal depth the iris normal and there was a flat ectropion uveae The lens was clear and elongated ciliary processes were noticed Behind the lens was an irregular greyish yellow tissue with superficial vessels and



Fig 3
Case 2 Age 1 year The right eye is shrunken

haemorrhages A grey detachment was seen peripherally in the retina The corneal diameter increased and by 7 months was 14 mm The intraocular tension was 37 mm The detachment was total and there was an incipient cataract At the age of 13 months the *intraocular tension* was 14 5/7 5 (*Schiotz*) and the corneal diameter 13 mm the anterior chamber was steep because the iris was retracted The cataract and the other features were unchanged (Fig 4)

Comment

This child had bilateral retinal non attachments observed at the fourth day of life The detachment soon became total During the first 2 years of life when psychomotor development was normal no definite diagnosis could be established because the family history was negative It was the occurrence of mental re tardation withdrawal and cerebral dysrythmia that indicated the diagnosis of Norrie s disease



Fig 4
Case ? Left eye age 1 year Irregular pupil elongated ciliary processes white cataract

Case 3

E F P b 26 VI 46 This boy was the oldest of four children and at his birth no relatives had been blind A brother of his maternal grandfather was mentally retarded but there was no indication of eye diseases The parents were not consanguinous Pregnancy was uneventful birth 4 weeks before expected term the birthweight was 2 500 g The infant was not given oxygen When he was 3 months old the mother had herpes zoster and 6–7 days later the boy had chickenpox He became blind before he was 5 months old but his psychomotor development was presumably normal until he was 2 years old He talked at 12 months walked at 24 months and was toilet trained at the age of 4 years He was admitted as a boarding pupil to the kindergarten at the National Institute for the Blind when he was 2 years old but 3 years later he was considered to be too retarded to remain there he then lived at home and at the age of 8 he talked fluently played with a good deal of imagination and moved easily all over the parents farm A neurological examination at the age of 21 months showed normal functions and a normal pneumo encephalogram The hospital

staff considered him retarded At the age of 8 years he was transferred to a mental hospital for further training and this was probably a very traumatic experience Two years after he had arrived he had lost most of his active language and the emotional contact with his mates and attendants was very weak His dementia progressed his language became unintelligible and at the age of 16 he began to tear his clothes and bed linen apart so he was fitted with gloves by night These spells subsided and at the age of 23 he was docile but was practically unoccupied He was found of music and his hearing was considered good A dental examination at the age of 23 years revealed early childhood rings on the crowns of all incisors and canines (B Russell D D S)

Eyes

White pupils were noted at the age of 5 months but the mother thought that blindness had already then been present for some time Both eyes presented flat anterior chambers atrophic irides and white retrolental opacities The



Fig 5

Case 3 Left eye There is a white inferior band shaped opacity and a yellow mature cataract



Fig 6
Case 3 Right eye The eye is shrunken the cornea is opaque with a band shaped white opacity

tension was raised in the left eye Metastatic uveitis and pseudo glioma were suspected At the age of 21 months there was atrophy of the retrobulbar tissue and both eyes were deeply set and shrunken Posterior synechiae and ectropion uveae were present in both eyes and the retrolental opacities were still evident At the ages of 5 and 6 years the corneae were clear but there were bilateral cataracts At 23 years of age the corneal diameters were 10 and 11 mm the corneae were opaque in the lower halves and there were anterior synechiae The right anterior chamber was shallow (Figs 5 and 6)

Comment

The clinical impression was that this boy had metastatic uveitis after chickenpox at the age of 3 months although the infection occurred some time before blindness was observed His infantile development was normal but he was retarded at the age of 21 months This retardation seems to have been mild until he was transferred to a mental hospital During this stay he was at some time fitted with gloves a treatment which must have had a very harmful influence on his tactile

orientation Eventually he became severely retarded and lost most of the abilities that he had had formerly Although he had infantile bilateral retrolental opacities without oxygen treatment a normal development until nearly 2 years of age and later a progressive dementia the diagnosis of Norrie s disease was only established when he had a brother with the same disease

Case 4

P A I P b 20 IV 64 brother of case 3 Pregnancy was *complicated* 6 weeks after the last period the mother was treated with Primulut® (norethisterone) 5 mg × 4 for 2 days During the first trimester she was given Moloid® (glyceryl nitrate mannityl nitrate) for migraine and during the last 2 months of preg nancy she had hypertension and oedema Birth occurred 1 month before ex pected term birth weight was 2 200 g but the child was well and not given oxygen At the age of 4 to 5 months vision decreased and at 5 months the child was blind

At the age of 6 months the boy had passed normal milestones but from this age to the age of 4½ years he was admitted to hospital six times (the eye disease, otitis media three times repairs of a testicular hydrocele and of an inguinal hernia) He spoke single words at the age of 2 years 9 months and at this age he had good spatial orientation while crawling He walked late and at the age of 4 years it was noted that his voice was remarkably low At this age he often moved round in circles waving a little scrap of paper in front of his face he avoided contact with everybody except his parents and sisters He found his way easily in the home and on the farm At the age of 6 years he went to kinder garten with sighted children and began slowly playing with them but sometimes he withdrew into himself and would then circle round and flap with his arms his voice became normal and he dressed and ate slowly without help

At the age of 7 he was still extremely slow but his reasoning and vocabu lary were within normal limits He started in a normal school one year late at the age of 8½ years and the impression was that of a child with a normal intellectual capacity but slow and passive He had an auxiliary teacher at his side throughout the first year and learnt to read and type Braille He was on a level with his classmates in arithmetic

Eyes

At 4 to 5 months of age the mother noticed a squint and decreased vision When he was 5 months old there was no perception of light He was examined at the local hospital

Right eye The cornea was clear the anterior chamber flat due to posterior synechiae The iris was atrophic and there were retrolental avascular masses The tension was 4/7 5 (Schiotz) This eye was enucleated on suspicion of retino blastoma

Left eye Only excessive myopia was found initially but at the age of 9 months this eye began to shrink and at 15 months of age retrolental proliferations were noted When he was 4½ years old there was slight phthisis the cornea was clear the anterior chamber flat the lens clear and avascular irregular retro lental masses were visible through the pupil

Microscopy of the right eye (Eye Pathology Institute 516/64) (Fig 7) The retina was totally detached and contained degenerative rosettes old haemor rhages gliosis and connective tissue proliferations Near the disc there was proliferation of the pigment epithelium Behind the detachment there were rem



Fig 7

Case 4 Histological section The cornea is normal the lens cataractous There are synechiae between the cornea and the atrophic iris and between the lens and the iris The fibrous vitreous is adherent to the lens and the iris It contains degenerative rosettes

orientation Eventually he became severely retarded and lost most of the abilities that he had had formerly Although he had infantile bilateral retrolental opacities without oxygen treatment a normal development until nearly 2 years of age and later a progressive dementia the diagnosis of Norrie s disease was only established when he had a brother with the same disease

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Fig 8

Case 5 Section of lens and vitreous In the upper half of the picture the cataract is seen The fibrous vitreous is adherent to the posterior part of the lens Degenerative rosettes and an abundancy of fibroblast proliferation is seen below The hyaloid artery is calcified and surrounded by haemorrhagic remnants

thelium was atrophic posteriorly and partially missing Anteriorly the vitreous was vascular and hyperplastic it contained haemorrhagic material and a calci fied persistent hyaloid artery The ciliary epithelium was proliferating and the ciliary processes elongated and adherent to the vitreous The choroid and the optic nerve were atrophic the lens cataractous The iris was atrophic and there was an ectropion uveae Broad peripheral synechiae covered the chamber angle but the trabeculae and Schlemm s canal were well developed though atrophic (Fig 8)

Comment

There is but little information on the early history of these two brothers Both were normal at birth and had bilateral detachments within a few weeks of life They became retarded in early childhood The histological preparation showed

Table II

Survey of pedigrees and cases of Norrie's disease published after the delineation of the disease Extension of previously reported pedigrees excepted

Authors	No of families	No of cases	Mental retardation	Histology
Franceschetti et al (1963)	1	2	1	–
Cook (1964)	1	2	–	1
Greer (196[illegible])	1	2	–	1
Hansen (1968)				
Nance et al (1969)	1	9	2	–
Ponte et al (1968)	1	5	4	–
Ricci (1969)	2	4	4	–
Lomickova et al (1969)	1	10	4/10	–
Blodi et al (1969)	1	8	1	2
Hamburg (1970)	1	4	1	2
Holmes (1971) Fradkin (1971)	1	6	2	–
Ashton (1971)	1	3	–	1
Peretz (1955 1971)	1	18	1	–
Klein (19[illegible])	1	4	2	–
Brini et al (1972)	1	7	–	1
Townes (1966 1973)	1	3	–	2
Bos (1973)	1	6	3	1
Apple et al (1974)	1	2	–	1
Present cases	4	6	5	3
	22	101	30/75*	15

* Mental development was estimated in 75 of the blind patients

layers have shown large vessels and abundant haemorrhage The ciliary processes have been elongated and the chamber narrow through the formation of synechiae

The ophthalmic differential diagnosis in infants include retinoblastoma (as in case 3) Coats disease juvenile retinoschisis which is also X linked autosomal recessive falciform detachment persistent hyperplastic primary vitreous retrolental fibroplasia metastatic endophthalmitis and massive retinal fibrosis In

patients who come for a diagnostic appraisal in later life the differential diagnostic problems include X linked microphthalmia and X linked cataract These differential diagnostic problems have been discussed extensively elsewhere (Warburg 1966)

Hearing

A number of the previously reported patients suffered from loss of hearing the ophthalmological reports are vague on this point but Warburg (1966) estimated that one third of the affected persons had a hearing defect This may appear as early as 4 months (Blodi et al 1969) and as late as 45 years (Warburg 1966) The severity is variable There are a number of syndromes in which deficient hearing and vision occur together (Bergsma 1972) but among them only Norrie's disease presents with bilateral retinal non attachment Differential diagnostic problems thus cannot be expected

Neuro-psychiatric signs

Of the 142 patients reported before the delineation of Norrie's disease mental development was described in 94 Of these 49 were mentally retarded Of the 101 patients reported after the establishment of this nosologic entity mentality was described in 75 and 30 of these were retarded (Tables I and II) While psychomotor development is usually normal during the first years of life Reichel (1960) and Ponte et al (1968) reported on patients with congenital mental retardation Dementia may set in at any time in life but a number of patients retain normal and even superior mentality throughout life (Warburg 1966) The patients with a late appearing dementia have shown psychotic features such as auditory hallucinations restlessness and destructive behaviour and the level of their intellectual functions is therefore difficult to estimate There is also in this disease a progressive degeneration of the central nervous system at work indicated by aquired grand mal and petit mal seizures

The psychiatric differential diagnosis is concerned with mental retardation and psychosis in congenitally blind males It is well known that autistic features are predominant in congenitally blind persons (Elonen & Zwarensteyn 1964) and that blindness is much more prevalent in mentally retarded individuals than in other persons (Warburg 1970) In Norrie's disease however blindness is always due to congenital malformation and detachment of the retina and the most important differential diagnostic problems in psychiatric patients are therefore concerned with retrolental fibroplasia and toxoplasmosis endophthalmitis It is thus important to know whether or not the child was treated with oxygen

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BACTERIAL FLORA OF THE NORMAL CONJUNCTIVA
II METHODS OF OBTAINING CULTURES

BY

J A FAHMY S MØLLER and M WEIS BENTZON

The methods used for obtaining bacterial cultures from the normal con junctiva were reviewed
A comparison was made between four methods *a*) platinum loop *b*) cal cium alginate swab *c*) dry and *d*) wet cotton wool swab using agar (all methods) and serum bouillon (methods *b c d*) as culture media The com parisons were based on the isolation rate of *Staphylococcus albus* and corynebacteria
At the same time four topical anaesthetics (benoxinate tetracaine pro paracaine cocaine) and one vital stain (tetrazolium - alcian blue mixture) instilled into the conjunctiva immediately before taking samples were studied for their effects on the isolation rate of *Staphylococcus albus* and corynebacteria
For isolating *Staphylococcus albus* method *d* was found to be superior to the others when the methods were used in the above mentioned order and when agar was the only culture medium used On the other hand after instillation of the above mentioned eyedrops causing the so called wash ing out effect (Fahmy et al 1974) method *a* was found better than the others providing the methods were used in the *a b c d* order and when agar only was used When considering the growth of *Staphylococcus albus* in serum boullion as well methods *b c* and *d* were found equal in effect but somewhat superior to *a* Corynebacteria showed nearly the same isola tion rate with all the methods before as well as after the instillation of eye drops

Key words conjunctiva – flora – normal – bacteria – cultivation methods – topical anaesthetics – vital stain

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The instillation of the above mentioned eye drops significantly increased the recovery rate of *Staphylococcus albus* whilst the incidence of coryne bacteria remained unchanged

Since the early days of ophthalmic bacteriology efforts have been made to find an effective technique for the isolation of bacteria from the eye

The *platinum loop* a basic appliance in every bacteriological laboratory seems to be one of the oldest instruments still in use for obtaining cultures from the conjunctiva (Fick 1887 Lawson 1898 Gradle 1910 Barfoed 1953 Smith 1954 Doden 1957 Makabe 1971 Fahmy et al 1974) The same is true for the *spatula* (Gifford 1898 Lindner 1921 Pillat 1921 Howard 1924 Lucic 1927 Rodin 1945 Barfoed 1953) and *cotton wool swab* which was used either *dry* (Fyre 1898 Edmund 1927 Keilty 1930 Cooper 1935 de Ocampo et al 1965 Glawogger 1969) or *moistened* with a nutrient broth (Imre 1911 Khorazo & Thompson 1935 Soudakoff 1954 Cason & Winkler 1954 Chang 1957 Locatcher Khorazo & Seegal 1972) Some authors introduced the broth directly into the conjunctival cul de sac and sucked it up again mixed with the conjunctival fluid by means of a sterile *pipette* (Imre 1911 Lowenstein 1911 Metafune & Albanese 1912 Lindner 1914 Kraupa 1914 Stanka 1924) Others (Elsching & Ulbrich 1909 McKee 1924) collected only the *tears* in sterile tubes Another unique method was that of Wissman (1924) who took a sample for the laboratory by inserting a sterile *silk thread* into the lower fornix and removing it shortly after moistened with the conjunctival secretion

Several authors (Fyre 1898 McKee 1924 Lucic 1927 Edmund 1927 Barfoed 1953 Soudakoff 1954 Muller 1957 Drewnoik & Wachtel 1967 Glawogger 1969) stated that the bacteriological results especially the incidence of sterile cultures were largely dependent on the technique used for obtaining material from the conjunctiva which in the present author s opinion is usually not sterile

Consequently various attempts have been made to compare some of the methods mentioned in order to find the most suitable

Eyre (1898) claimed that the cotton wool swab was superior to the platinum loop This was confirmed by Lundsgaard (1925) who tested these methods together with the pipette Others (Elsching 1926 Kuffer & Schneider 1926) compared the pipette with the spatula (scraping technique) and found that the former technique was better than the latter Khorazo & Thompson (1935) stated that by introducing broth into the conjunctival sac and sucking it back gave more chance of contamination and gave no more positive cultures than the moistened cotton wool swab which was later found (Locatcher Khora a & Seegal 1972) to be more effective than the dry one In his thesis on *Conjunctivas Bacteria Flora* Barfoed (1953) found that the loop was more suitable than the cotton swab

Thus in reviewing the literature a contradictory conception could be demonstrated and no single method could be denoted as more superior This fact is seen in Table I which correlates the incidence of negative cultures with the different methods used by the various authors

The present study was undertaken primarily to compare some of the above mentioned methods (platinum loop dry and wet cotton swab) together with another kind of swab (calcium alginate) This swab has not been used previously in ophthalmology but in other studies on the isolation of streptococci from the throat etc. (Higgins 1950 Cain & Steele 1953 Taplin & Lanstell 1973) it proved to be better than ordinary cotton swabs Furthermore the study was undertaken in order to investigate the influence of four topical anaesthetics (0 2 % benoxi

Table I
Incidence of sterile cultures as related to the methods used by the different authors

Study	Number of cases	Sterile cultures %	Method
Lawson (1898)	200	20	loop
Fyre (1898)	150	50	dry cotton swab
Lindner (1914)	500	10	pipette
Kraupa (1914)	635	33	pipette
Stanka (1924)	412	15	pipette
Edmund (1926)	86	0	dry cotton swab
Lucic (1927)	100	0	spatula (scraping)
Keilty (1930)	100	43	dry cotton swab
Cooper (1935)	92	6.5	dry cotton swab
Khorazo & Thompson (1935)	1112	17	wet cotton swab
Rodin (1945)	42	20	spatula (scraping)
Barfoed (1953)	501	44.5	loop
Smith (1954)	5000	47	loop
Soudakoff (1954)	3000	21	wet cotton swab
Cason & Winkler (1954)	1604	93	wet cotton swab
Chang (1957)	100	9	wet cotton swab
Doden (1957)	915	8 7	loop
Glawogger (1969)	150	14 7	dry cotton swab
Makabe (1971)	683	66	loop
Locatcher Khorazo & Seegal (1972)	1641	0	wet cotton swab

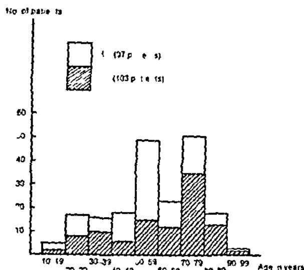


Fig 1
Age and sex distribution of 200 patients examined

nate 1% tetracaine 0.5% proparacaine 2% cocaine) and the vital stain tetrazolium alcian blue (Norn 1972 a) on the isolation rate of bacteria as well as on the number of colonies recovered from the conjunctiva

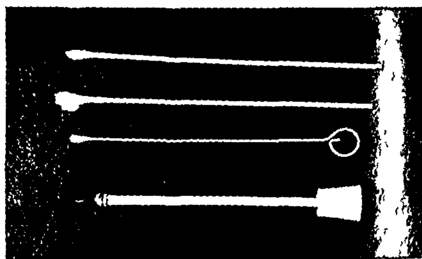


Fig 2
Instruments used for the isolation of bacteria *from* the conjunctiva (from bottom)
a) Platinum loop *b)* Calcium alginate swab *c)* Dry cotton wool swab
d) Wet cotton wool swab

Material and Methods

The material comprises 200 patients selected from the out patients of the Eye Department of Kommunehospitalet Copenhagen It was made certain that none of the patients had any signs of eye inflammation or misfunction of the lacrimal apparatus and that none had received antibiotics or corticosteroids in recent weeks The age and sex distribution of the material can be seen from Fig 1

Sampling methods The material of 200 patients was divided into five groups of 40 patients each in the order of their admittance Four samples from the lower fornical and *tarsal conjunctiva of each patient were taken successively by means of a)* calibrated platinum loop (~one microliter) *b*) calcium alginate swab *c*) dry cotton wool swab *d*) wet (0.9 % sodium chloride) cotton wool swab (Fig 2)

The same procedure (taking another four samples from the same eye) was repeated immediately after the application of the following sterile eye drops (one kind for each group of 40 patients) 1) 0.2 % benoxinate hydrochloride (without preservatives) 2) 1 % tetracaine hydrochloride (without preservatives) 3) tetrazolium alcian blue mixture (without preservatives) 4) 0.5 % proparacaine hydrochloride (with benzalkonium chloride and 0.2 % chlorobutanol as preservatives) 5) 2 % cocaine hydrochloride (without preservatives)

Table II
Incidence of microorganisms cultured from 200 patients

Microorganisms		*Incidence in percent*
Staphylococcus albus		97.5
Corynebacteria		71
Staphylococcus aureus		10
Streptococcus		6.5
haemolyticus	0.5	
non haemolyticus	2	
faecalis	4	
Gram negative bacilli		3
Esherishia coli	0.5	
Pseudomonas aeruginosa	0.5	
Klebsiella pneumoniae	0.5	
Proteus morgani	0.5	
Bacterium anitratum	0.5	
Enterobacter cloacae	0.5	
Micrococci		3
Diplococcus pneumoniae		0.5
No growth		none

Thus eight samples from each patient were taken four before and another four after the instillation of one of the mentioned eye drops

Bacteriological methods The samples were immediately inoculated on 5 % horse blood agar plates and incubated (37 C) for 48 hours For the methods b c and d the swabs were further inoculated and kept in serum bouillon tubes for 48 hours (37°C) to observe any bacterial growth not occurring primarily on the agar plates

The identification of bacteria was undertaken by ordinary laboratory methods similar to those described by Cowan & Steel (1965)

Statistical methods The results were analysed at the Department of Bio Statistics Statens Seruminstitut Copenhagen The statistical methods used were largely the same as described previously (Fahmy et al 1974 a) i e the observations were divided into four groups according to the number of colonies (0 1–5 6–25 and > 25) For methods b c and d the zero group could be divided according to the results obtained in bouillon. Pairwise comparisons were carried out between the results before and after the instillation of eye drops recording a difference if the two observations fell into different groups Here a distinction was made between (a) 0 versus > 0 and (b) cases where both results were positive The statistical test used was the McNemar test (Armitage 1971) For the comparisons between the four methods all 16 combinations of positive and negative results were considered using χ test for the comparisons carried out

Results

Incidence and flora

Before the instillation of any eye drops the flora of the 200 patients was as follows 53 patients (26 5 %) showed one kind of bacteria 121 two kinds (60 5 %) while 26 had three or more kinds (13 %) None of the patients showed a sterile conjunctiva One hundred and eighty five patients (92 5 %) had *Staphylococcus albus* (coagulase negative) 142 (71 %) corynebacteria 20 (10 %) *Staphylococcus aureus* (coagulase positive) 13 (6 5 %) streptococci 6 (3 %) gram negative bacilli 6 (3 %) micrococci and 1 (0 5 %) *Diplococcus pneumoniae* (Table II)

Comparison between the four methods

The comparisons were based on the isolation rate of *Staphylococcus albus* and corynebacteria only as the incidence of other bacteria (*Staphylococcus aureus* streptococci gram negative bacilli) was too low to permit any sufficient statistical analysis

Fig 3 shows the isolation rate of *Staphylococcus albus* as detected by the four methods before and after instillation of the above mentioned eye drops

Out of a total number of 128 patients with *Staphylococcus albus* (growth only

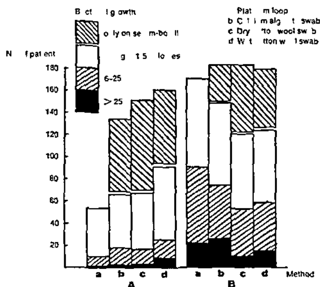


Fig 3
Isolation rate of *Staphylococcus albus* as detected by four methods A) before and B) after the instillation of eye drops (benoxinate tetracaine proparacaine cocaine and tetrazolium alcian blue vital stain)

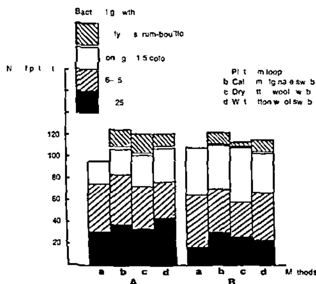


Fig 4
Isolation rate of corynebacteria as detected by four methods A) before and B) after the instillation of eye drops (benoxinate tetracaine proparacaine cocaine and tetrazolium alcian blue vital stain)

on agar and *before the instillation of eye drops*) method *a* revealed 54 patients method *b* 66 method *c* 67 and method *d* 91 patients (Table III)

Thus none of the methods alone could reveal all the cases with *Staphylococcus albus* However method *d* seemed the most favourable being the only positive in 24 cases with *Staphylococcus albus* while the other methods were negative and *d* was negative in four instances while the others were positive (Table III) (the incidences in the last column of Table III are significantly different χ = 17 $P = 0\ 0008$) When considering the growth of *Staphylococcus albus* in serum bouillon as well (total 185 patients) method *b* revealed 134 method *c* 152 and method *d* 160 (Fig 3)

After the instillation of the previously mentioned eye drops causing a transfer of bacteria from the upper to the lower parts of the conjunctiva (Fahmy et al 1974) a total number of 188 patients with *Staphylococcus albus* (growth only on agar) could be found

The isolation rate of the four methods was 170 148 120 and 123 respectively This made method *a* the most suitable a fact which was further confirmed from Table III (positive alone in 12 cases and once negative while the others were positive ($\chi^2 = 18\ 5$ $P = 0\ 0004$)

When considering the growth of *Staphylococcus albus* in serum bouillon as well (total 194 patients) we find that methods *b* *c* and *d* with an isolation rate of 182 182 and 178 respectively became more equal to each other and significantly superior to method *a* with 170 isolations ($\chi = 14\ 8$ $P = 0\ 0022$)

For growth on agar only the above mentioned conditions for *Staphylococcus albus* were not similar to those found for corynebacteria which showed nearly the same isolation rate with all the methods before as well as after the instillation of eye drops (Fig 4 and Table IV) The isolation rate for the four methods (growth on agar for method *a* and agar + serum bouillon for *b* *c* *d*) was 96 125 121 and 121 (total 142) and after eye drops 108 122 113 and 115 respectively (total 132)

Influence of eye drops on isolation rate and number of colonies

The study of the influence of the previously mentioned eye drops on (a) the isolation rate and (b) the number of colonies of *Staphylococcus albus* and coryne bacteria was carried out by only one of the methods in order to facilitate the comparisons Method *a* was chosen since this method is well defined (precisely calibrated platinum loop = one microliter)

From Table V it is seen that the isolation rate of both microorganisms was influenced differently after the instillation of eye drops whilst *Staphylococcus albus* showed highly significant increases ($P < 0\ 0001$ to $0\ 0074$) the isolation rate of corynebacteria remained nearly the same

Table V

Effects of five different eye drops on the isolation rate of *Staphylococcus albus* and corynebacteria

Microorganisms	Eye drops	Bacterial growth					McNemar's test Level of significance
		Before eye drops / After eye drops	No growth / No growth	Growth / No growth	No growth / Growth	Growth / Growth	
S albus	Benoxinate hydrochloride		3	1	24	12	$P < 0.0001$
	Tetracaine hydrochloride		5	0	29	6	$P < 0.0001$
	Tetrazolium alcian blue		11	3	15	11	$P = 0.0074$
	Proparacaine hydrochloride		6	0	26	8	$P < 0.0001$
	Cocaine hydrochloride		1	0	26	13	$P < 0.0001$
Corynebacteria	Benoxinate hydrochloride		24	0	4	12	*n s
	Tetracaine hydrochloride		22	2	3	13	*n s
	Tetrazolium alcian blue		12	3	5	20	*n s
	Proparacaine hydrochloride		17	2	5	16	*n s
	Cocaine hydrochloride		8	2	4	26	*n s

*n s = not significant

Table VI

Effects of five different eye drops on the number of colonies of *Staphylococcus albus* and corynebacteria

Microorganisms	Eye drops	Number of colonies (1- > 25)				McNemar's test Level of significance
		Before eye drops After eye drops	before < after	before > after	equally positive	
S albus	Benoxinate hydrochloride		6	0	6	$P < 0.05$
	Tetracaine hydrochloride		3	2	1	*n s
	Tetrazolium alcian blue		3	1	[illegible]	*n s
	Proparacaine hydrochloride		6	0	12	$P < 0.05$
	Cocaine hydrochloride		7	1	5	$P < 0.05$
Corynebacteria	Benoxinate hydrochloride		2	4	6	*n s
	Tetracaine hydrochloride		1	8	4	$P < 0.05$
	Tetrazolium alcian blue		1	10	9	$P < 0.05$
	Proparacaine hydrochloride		2	6	8	*n s
	Cocaine hydrochloride		8	7	11	*n s

*n s – not significant

However matching only the positive cases (Table VI) before and after eye drops (before < after and before > after) a significant decrease was found in the number of colonies of corynebacteria when tetracaine and tetrazolium alcian blue mixture were used ($P < 0 05$) while no effect on *Staphylococcus albus* could be observed Benoxinate proparacaine and cocaine eye drops showed a reversed effect by increasing the colonies of *Staphylococcus albus* ($P < 0 05$) while not affecting those of corynebacteria

Discussion

Is the normal conjunctival ac ever sterile? This question has occupied the minds of many authors throughout the years Already at the end of the last century it had been answered by several observers (cited by Lawson 1898) who did not believe in the sterility of the healthy conjunctiva

However various investigators (Table I) including Lawson himself (1898) have encountered sterile conjunctivas in normal patients Few authors (Edmund 1927 Lucic 1927 Locatcher Khorazo & Seegal 1972) could find bacteria in every case examined

Quite early on (Eyre 1898) it seemed clear for many (McKee 1924 Lucic 1927 Edmund 1927 Barfoed 1953 Soudakoff 1954 Mull r 1957 Drewnoik & Wachtel 1967) that the incidence of negative results was largely dependent upon the methods used for isolating bacteria from the conjunctiva

Some investigators (Lucic 1927 Muller 1957 Rathmann 1963 Drewnoik & Wachtel 1967 Glawogger 1969) emphasized the importance of other factors such as the kinds of culture media used and the time elapsing until their ino culation.

The present study demonstrated that for the isolation rate of *Staphylococcus albus* the wet cotton wool swab (method *d*) was superior to the other methods if agar was the only medium used for cultivation and if the methods were used in the *a b c d* order By so doing method *d* was probably favoured as so much more material was produced by the preceding methods giving increased lacrima tion and desquamated epithelial cells On the other hand after the instillation of eye drops into the conjunctiva causing the washing out effect described pre viously (Fahmy et al 1974 a) which transferred bacteria from the upper to the lower parts of the conjunctiva the platinum loop (method *a*) when used first in the order of methods was found to be the most effective

This could probably be due to the fact that this method was used here as number one directly after the instillation of eye drops and there was more material

However when the growth of *Staphylococcus albus* was considered on agar and after incubation in serum bouillon the loop was found to be somewhat less effective than the other methods which in this case were more equal

As for the isolation rate of corynebacteria all methods seemed to be equally good both before and after the instillation of eye drops

When carrying out the present study no attempt was made to compare the results with other studies since no one else had used anaesthetic eye drops before obtaining cultures

In 1966 an important laboratory study (Kleinfeld & Ellis) was undertaken to determine what effect topical anaesthetics used in ophthalmology might have upon the growth of microorganisms since material for cultures especially from the cornea or the conjunctiva postoperatively is usually obtained after the application of a topical anaesthetic Kleinfeld & Ellis examined *in vitro* the effect of four anaesthetics (benoxinate tetracaine proparacaine cocaine) and two preservatives (chlorobutanol butyl parahydroxy benzoate) on the growth of three test organisms (*Staphylococcus albus P aeruginosa C albicans*) and found that all the anaesthetics (commercially available) and preservatives inhibited the growth of all the organisms in varying degrees

The present investigation attempted to evaluate the mentioned results clinically by studying the effects of the same anaesthetics on the isolation rate of two microorganisms mostly found in the normal conjunctiva namely *Staphylococcus albus* and corynebacteria

The results were surprising as the isolation rate of *Staphylococcus albus* showed a highly significant increase ($P < 0.001$) even after the instillation of tetrazolium alcian blue mixture (vital stain) known for its toxic effect on living cells (Norn 1972 b) The isolation rate of corynebacteria was not influenced

Previously Fahmy et al (1974) demonstrated that the instillation of benoxinate eye drops immediately before obtaining cultures altered the topographical distribution of *Staphylococcus albus* and corynebacteria on the conjunctiva to a preponderance in its lower parts This was explained by a reflex outflowing of the tears which together with the eye drops helped to wash mucus and desquamated epithelial cells (with bacteria) out of the eye

Both phenomena are probably equally responsible for the increase of the isolation rate of *Staphylococcus albus* seen in the present study

A different relationship between *Staphylococcus albus* and corynebacteria to the conjunctival cells (living and desquamated) may explain the unchanged incidence of corynebacteria after the instillation of eye drops Smith (1954) stated that corynebacteria was known to be more adherent to the conjunctival epithelium while Pillat (1921) found that *Staphylococcus albus* was closely related to the desquamated surface cells

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SYMPTOMS SIGNS AND EARLY COURSE OF OPTIC NEURITIS

BY

EEVA NIKOSKELAINEN

The study was an analysis of the early course of optic neuritis based on the case histories of 185 patients 57 % of whom were females and 43 % males More than half of the patients suffered from multiple sclerosis In 28 % of the patients the etiology remained unknown
The most common initial symptom was acute decrease in visual acuity but in 25 % the onset was subacute or slow Pain occurred in 62 % and preceded decrease in visual acuity in 16 % of the cases
The initial attack was unilateral in 70 % and bilateral in 30 % of the patients On admission in 64 % of the involved eyes visual acuity was poor and in 73 % the defect in the visual field involved the central field
The optic disc was normal in 46 % blurred and/or hyperaemic in 20 % oedematous in 23 % and in 11 % there was temporal or total pallor already on admission The last finding was common in patients with bilateral optic neuritis with a slow onset
Six months after admission visual acuity was good or excellent in 56 % and the visual field was normal in 45 % of the involved eyes

Key words optic neuritis – optic neuropathy – optic atrophy – multiple sclerosis – polyneuropathy – visual fields – papilloedema – pupillary function – ocular pain

The term optic neuritis refers to the involvement of any part of the optic nerve in a disease process whether inflammatory vascular or degenerative which impairs nerve conductivity as indicated by visual field changes (Carroll 1956

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Walsh & Hoyt 1969) The most common cause is a plaque of demyelination in the optic nerve in multiple sclerosis (MS) Involvement of the optic nerve may also occur in various infections by direct spread from adjacent structures There are however involvements of the optic nerve in which the process is not infective as the term optic neuritis would seem to imply The term optic neuropathy in these cases better describes the process in the optic nerve caused by disturbed nutrition mediated by an abnormal biochemical or vascular environment Common manifestations of this type are ischaemic optic neuropathy and nutritional amblyopia in association with chronic alcoholism (Foulds 1970) Leber s hereditary optic atrophy is commonly included as the cause of optic neuritis because the early picture is optic neuritis (Duke Elder & Scott 1971 Walsh & Hoyt 1969) Although there are several known causes of optic neuritis in 30–75 % of the cases according to the literature the etiology remains unknown (Marshall 1950 Oksala 1964 Rucker 1956)

The diagnosis of unilateral optic neuritis if associated with typical symptoms and signs is easy Sometimes the symptoms are however atypical Bilateral involvement especially if the optic discs are oedematous can present problems in differential diagnosis Rapid recovery is one of the characteristics of optic neuritis This is why the follow up period of patients is often short and the later case history of the patients remains unknown

The purpose of the present study was to make an analysis of the symptoms signs and early course of optic neuritis in 185 patients seen at the Department of Ophthalmology University of Turku during the years 1950–1974 and followed up by the author

Material

Based on hospital records there were 201 patients with optic neuritis seen in 1950–1969 at the Department of Ophthalmology (Table I) It appeared however that the initial diagnosis of optic neuritis was incorrect in seven patients Patients were invited to a follow up examination in which four more patients were rejected because the case history and re examination made the diagnosis of optic neuritis most unlikely In addition to this 28 patients were excluded because of insufficient data Twelve of the patients have died Thirty three patients refused to take part in re examination or they could not be traced Thus there were 117 re examined patients with optic neuritis All except one of these patients have been neurologically re examined (Nikoskelainen & Riekkinen 1974)

Table 1

Patients with optic neuritis treated at the Department of Ophthalmology University of Turku in 1950–1969

	No
1 *Other diagnoses*	11
cerebral tumour 3	
tumour of the nose 1	
aneurysma of the ophthalmic artery 1	
juxtapapillar chorioditis 2	
myopic degeneration 1	
serous detachment of the macula 1	
toxic amblyopia induced by methyl alcohol 1	
homonymous hemianopsia caused by circular insufficiency of the brain 1	
B Insufficient data	88
C Patients with optic neuritis	102
1 Dead 12 patients cause of death was	
multiple sclerosis 2	
encephalomyelitis 1	
malignant tumour in intestine 2	
pneumonia and stenocardia 1	
pneumonia and arteriosclerosis 1	
cirrhosis hepatis caused by *alcoholism 1*	
uraemia and polyneuropathy 1	
suicide 1	
myocardial infarction 1	
congestive heart failure 1	
2 Refused the re examination or could not be traced 33	
3 Re examined patients with optic neuritis [illegible]	
Total	201

This study also includes 68 *patients with optic neuritis* who have been followed up with repeated eye examinations by the author during the years 1970–1973 These patients have been neurologically examined at least during the attack of *optic neuritis*

Methods

All patients were interviewed carefully for ophthalmological and neurological symptoms as well as for possible medical treatments The symptoms and signs registered on hospital files were studied The eye examination on patients performed by the author included determination of visual acuity in both eyes with E hooks in lines Visual acuity was expressed in decimal numbers and was considered excellent if V > 1 25 good if V = 0 8–1 25 fair if V = 0 4–0 7 and poor if V ≤ 0 3 The pupils were inspected for size shape equality and reaction to light stimulation The visual fields were examined with the Goldmann kinetic perimeter For the investigation of relative defects in the central field the test object which was noticed by the patient at 20 from the fixation point was used If there were defects in the visual fields they were mapped accurately by changing the test object and the intensity In the examination of visual acuity and visual fields the glass correction for refraction and possible presbyopia was used if necessary Intraocular pressure was determined with an applanation tonometer in the Haag Streit microscope in all patients treated during the years 1970–1973 at least on admission and in re examined patients if the age exceeded 40 years The ophthalmoscopy was performed through the pupils which had been dilated with tropicamide or cyclopentolate drops Examination of the eyes included the study with the Haag Streit biomicroscope including the study of eye fundus using the Goldmann three mirror contact glass

The colour vision was not systematically studied but the tests with the pseudoisochromatic plates of Bostrom C G & Kugelberg I and Farnsworth Panel D 15 were occasionally performed In some patients the Farnsworth Munsell 100 hue test and the Goldmann static perimetry was performed by Dr Terttu Karo M D in order to clarify the diagnosis of optic neuritis

Results

Age and sex The material comprised 105 (57 %) females and 80 (43 %) males The age and sex distribution at the onset of the disease is shown in Table II Most of the patients were young or middle aged the mean age being 32 ± 12 years

Etiology (Table III) More than half of the patients suffered from MS Twelve of the patients had polyneuropathy with bilateral optic neuritis One of these patients suffered from subacute combined degeneration one from uraemia caused

Table II

Age and sex distribution of 185 patients with optic neuritis Age on admission to hospital during the initial attack of optic neuritis

Age in years	Female No	Male No	Total No	Per cent	
0– 9	2	1	3	2	
10–19	9	8	17	9	
20–29	43	25	68	37	81 % (20–49)
30–39	23	26	49	26	
40–49	20	14	34	18	
50–59	5	4	9	5	
60–69	1	2	3	2	
> 70 years	2	0	2	1	
	105	80	185	100	

by nephritis and the others were chronic alcoholics Eight patients with a vascular cause of optic neuritis had other manifestations of disturbed blood circulation along with ischaemic optic neuropathy Only two of these patients were younger than 50 years the first of whom had suffered from rheumatic fever complicated by mitral stenosis and the other from three myocardinal infarctions

Six patients with Leber's hereditary optic atrophy belonged to the same family In the family of the seventh patient there were other males with this disease (Elenius 1967)

Infectious etiology was found in five patients Two had meningitis complicated by bilateral optic neuritis According to consultations an infectious cause was also suggested in two patients with upper respiratory infection preceding bilateral optic neuritis In both cases there was a full recovery and after a follow up period of 21 and 8 years no other neurological symptoms had appeared The unilateral papillitis of a 54 year old man was considered to be caused by purulent bilateral maxillary sinusitis

This material included two patients with the Guillain Barre syndrome The case history of one of these patients has been published elsewhere (Nikoskelainen & Riekkinen 1972)

The other patient a 22 year old woman had a short period of blurred vision in her right eye in May 1971 This symptom was preceded by an upper respiratory infection She did not consult a physician because the symptom disappeared within a couple

of days Soon after this she suffered from an acute ascending myelitis later followed by respiratory arrest She was treated at the Department of Neurology University of Turku and on the basis of the case history as well as the neurological and cerebrospinal fluid findings the Guillain Barre syndrome was diagnosed She made a good recovery during the subsequent months In March 1972 after a relatively symptom free interval she got acute retrobulbar neuritis in her right eye There was pain on eye movements and tenderness on palpation of the involved eye On admission the visual acuity in her right eye was hand movements The pupil reacted sluggishly to light and Gunn s pupillary sign was positive. The patient did not pass the tests with pseudoisochromatic plates and Panel D 15 with her right eye In the right visual field a large, absolute *central defect breaking into the periphery was demonstrated (Fig 1) The eye fundus*

Table III

The cause of optic neuritis in 185 patients with optic neuritis

Diagnosis	Patients treated during the years 1950–1969 who have been neurologically re examined* No of patients	Patients treated during the years 1970–1973 Diagnosis is based on neurological consultation No of patients	Total	
			No of patients	Per cent
Probable or possible multiple sclerosis	77	22 (6)	99	53
Polyneuropathy	3	9	12	7
Vascular	2	6	8	4
Leber s hereditary optic atrophy	6	1	7	4
Infectious	4	1	5	3
Guillain Barre syndrome	0	2	2	0 5
Unknown	25	27 (43)**	52	28
Total	117	68	185	100

one patient refused the neurological re examination but has been examined in a neurological clinic and diagnosed as having possible MS The follow up period of patients was from 3 to 24 years

** numbers in brackets show the size of the groups based on the diagnosis during the first visit to the clinic So far 16 patients according to the later course have been changed from the group with unknown cause to the group of MS patients

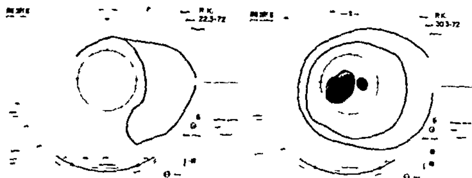


Fig 1
Visual field in unilateral optic neuritis in a woman with the Guillain Barre syndrome 1972-03-22 visual acuity in the right eye for hand movements 1972-03-30 visual acuity in the right eye for finger counting at 1 metre

was normal All tests on the left eye were completely normal The patient made a good recovery in the following weeks Figs 1 and 2 demonstrate the recovery of the visual field in the involved eye After a follow up period of 2.5 years visual acuity in the right eye was 1 5 in the left eye 2 0 The visual fields were normal The right optic disc was pale the left normal The patient was in good general health

Symptoms One hundred and thirty eight patients (75 %) came to hospital within 2 weeks after the onset of the disease Five patients had suffered from symptoms for more than 3 months before admission and three could not remember the interval between the onset of symptoms and admission which was not registered on the hospital files

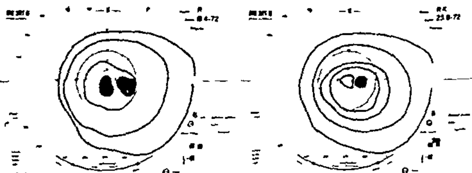


Fig 2
Visual field in recovery phase in unilateral optic neuritis (The visual field from the same eye as in Fig 1) 1972-04-18 visual acuity in the right eye 1 25 1972-08-23 visual acuity in the right eye 1 5

Table IV

The first symptom in the initial attack of optic neuritis in 185 patients

		No of patients	Per cent
1	Decrease in visual acuity (isolated)	107	58
2	Ache around or behind the involved eye (isolated)	2	1
3	Pain on eye movements (isolated)	7	4
4	Symptoms 2 and 3 together	20	11
5	Symptoms 1 2 and 3 together	37	20
6	Headache (isolated)	12	6
	Total	185	100

The commonest initial symptom was acute decrease in visual acuity often associated with pain in the eye region (Table IV) Pain preceded decrease in visual acuity in 29 (16 %) of the patients Some of these patients came to hospital because of pain in the eye region and decrease in visual acuity took place later Table V shows the time in which decrease in visual acuity reached its maximum Mostly there was a rapid dropping in visual acuity 27 (15 %) noticed decreased vision after waking in the morning In 33 (18 %) patients the onset was slow and

Table V

The time at which subjective decrease in visual acuity reached its maximum in 185 patients during the initial attack of optic neuritis

		No of patients	Per cent
1	Within some hours	53	29
2	Within 1–2 days	38	20
3	Within 3–7 days	43	23
4	Within 1–2 weeks	13	7
5	Slow gradual decrease in visual acuity	33	18
6	No subjective decrease in visual acuity	2	1
7	Unknown	3	2
	Total	185	100

Table VI

Visual acuity in the affected eyes on admission 2 weeks 1 month 2 months and 6 months later

	Visual acuity	On admission		2 weeks later		1 month later		2 months later		6 months later	
		No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent
Excellent or good	> 1 25	13	5	38	20	42	28	53	31	51	41
	0 8–1 25	28	12	45	23	43	28	48	28	19	15
Fair	0 4–0 7	45	19	25	13	11	7	18	10	9	7
Poor	0 1–0 3	48	20	26	14	20	13	14	8	14	11
	count fingers or less	106	44	58	30	37	24	40	23	32	26
	Total	240	100	192	100	153*	100	173	100	125*	100

the number of eyes has decreased because some patients refused to take part in the follow up

gradual Thirty patients were first seen in hospital when vision was already improving

One hundred and fifteen (62 %) patients complained of pain during optic neuritis in or behind the involved eye in 39 (21 %) it occurred only with eye movements Headache in the involved eye region was reported by 40 (22 %) and generalised headache by 24 (13 %) patients Uhthoff's sign a transient reduction of visual acuity during exercise or increased body temperature occurred in 37 (20 %) patients Seventy nine (43 %) noticed a subjective defect in their visual field and eight (4 %) had subjectively disturbed colour vision

Distribution of the lesions The initial attack of optic neuritis was unilateral in 130 (70 %) patients Bilateral optic neuritis occurred in 55 (30 %) of whom six had unilateral disease on admission but the other eye was also involved within 1 month

Visual acuity (Table VI) In most eyes there was a remarkable decrease in visual acuity of the affected eye on admission in 37 only hand movements or less were seen Visual acuity was however excellent or good in 41 eyes Some of these presented cases with mild disease In some patients visual acuity had already started to improve Furthermore in some patients the initial symptom was pain in the region of the involved eye or the cause of admission was optic neuritis in the other eye but the decrease in visual acuity took place later One month after admission already more than half of the eyes had good or excellent visual acuity Six months after admission visual acuity was excellent in 41 % good in 15 % fair in 7 % but poor in 37 % of the involved eyes

Visual field Table VII shows the findings in the visual fields of all involved eyes on admission In 174 (73 %) eyes the defect affected the central field The scotoma was centrocaecal in 60 eyes in eight eyes the central defect was associated with peripheral constriction of the visual field In 13 eyes the visual field was normal on admission In some of these cases the initial symptom and cause of admission was pain in the eye region There were also patients with bilateral optic neuritis in whom the one eye was normal or showed only oedema in the optic disc on admission but other symptoms of optic neuritis appeared later In one male patient a large central scotoma in the left eye was associated with a relative peripheral defect in the visual field in the right eye

In 23 eyes the visual fields seemed unreliable There had ben a discrepancy between the normal visual field and a marked reduction in visual acuity In some cases the visual fields had been studied by only using a large object and intensity and thus the relative defects could not be detected Some of the youngest patients did not co operate in the examination In all these cases other typical symptoms and the later course of the disease suggested a diagnosis of optic neuritis

Table VII

The visual fields in 240 involved eyes in 185 patients on admission

	The visual field	No of eyes	Per cent	
Central defect	Could not be studied because of amaurosis	12	5	73 %
	Absolute defect affecting the central and peripheral field	30	13	
	Absolute central or centrocaecal scotoma	89	37	
	Relative central or centrocaecal scotoma	43	18	
Paracentral or peripheral defect	Paracentral scotoma	9	4	8 %
	Peripheral defect alone	3	1	
	Enlarged blind spot and/or nerve fibre bundle defect	8	3	
	Normal	13	5	
	Unreliable	23	10	
	Not studied	10	4	
	Total	240	100	

Recovery in the visual fields was followed up by the author in patients seen during the years 1970–1973 (Table VIII) On admission 84 % of the involved eyes had a central defect Six months after admission 30 % of the eyes still had a relative or absolute central defect but in 45 % the visual field was normal

Ophthalmoscopical findings (Table IX) Most of the involved eyes had normal optic disc on admission indicating retrobulbar neuritis Fifty four eyes (23 %) presented a picture of papillitis with oedematous optic disc In three eyes papilloedema was associated with prepapillary haze and in two eyes with a star figure in the macula In a relatively high number of eyes (20 %) the optic disc was blurred and/or hyperaemic thus presenting a borderline type between retrobulbar optic neuritis and papillitis

In eleven eyes the macula showed slight disintegration The veins around the optic disc were dilated in 33 eyes in 14 eyes there were variations in the calibre

Table VIII

The visual field in the affected eyes on admission 2 weeks 2 months and 6 months later[1]

	Visual field	On admission		2 weeks later		2 months later		6 months later	
		No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent
Central defect	Could not be studied because of amaurosis	6	6	0	0	1	1	1	1
	Absolute central defect +/- peripheral defect	55	54	39	41	21	23	14	17
	Relative central defect	25	24	19	20	16	18	10	12
Paracentral or peripheral defect	Paracentral scotoma	7	7	9	10	3	3	5	6
	Enlarged blind spot +/- nerve fibre bundle defect	4	4	8	9	15	16	16	19
	Peripheral defect only	1	1	1	1	0	0	0	0
	Normal	4	4	18	19	34	37	39	45
	Could not be studied because of *extreme disability in patient with MS*	*0*	*0*	*0*	*0*	2	2	0	*0*
	Total	1021	100	941+	100	921+2	100	841+2	100

[1] only the visual fields studied by the author with Goldmann s perimeter have been included
[2] the number of eyes has decreased because some patients refused to take part in the follow up

Table IX

The appearance of the optic disc in the affected eyes on admission 2 weeks 2 months and 6 months later*

Optic disc	On admission		2 weeks later		2 months later		6 months later	
	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent
Normal	111	46	82	43	71	41	52	42
Blurred and/or hyperaemic	44	18	40	21	14	8	2	1
Blurred with haemorrhages around the optic disc	5	2	9	5	2	1	3	2
Papilloedema	54	23	30	16	20	11	0	0
Temporal pallor	24	10	23	12	50	29	35	28
Slight total pallor	2	1	7	2	15	8	11	9
Total pallor	0	0	1	0 5	3	2	22	18
Total	240	100	192*	100	175*	100	125*	100

* the number of affected eyes has decreased because some patients refused to take part in the follow up

of the retinal arteries Periphlebitis retinae occurred in three and cells in the vitreous body in seven affected eyes No abnormalities in the refracting media or fundus however could have been the cause of decreased visual acuity

Six months after admission oedema was no longer visible in the optic disc of any of the eyes but in more than half of the eyes there was temporal or total pallor in the optic disc

Other findings The colour vision was tested in 74 eyes With the exception of two eyes all (97 %) *had a disturbed red green colour vision* The pupillary function in 182 involved eyes showed the following abnormalities 45 eyes (25 %) had a slow reaction to direct light stimulation two pupils (1 %) did not react to direct light at all Gunn s pupillary sign was positive in 32 (44 %) out of 72 eyes studied with this test Anisocoria occurred in 18 (14 %) out of 131 eyes in which the pupil of the involved eye according to hospital files *had* been compared with that of the other eye In all of these eyes except one *the*

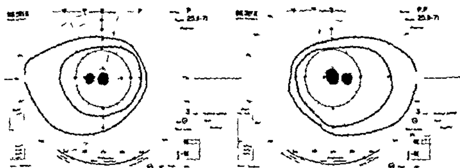


Fig 3

Visual fields in a 37 year old man with nutritional amblyopia caused by chronic alcoholism Visual acuity for finger counting in both eyes After one month s treatment with multivitamins full recovery final visual acuities in both eyes 1 5

pupil of the affected eye was larger than that of the healthy eye Tenderness on palpation was reported in 36 (43 %) out of 84 eyes according to hospital files

Bilateral optic neuritis Eighteen (33 %) of 55 patients with bilateral optic neuritis were females and 37 (67 %) were males The causes of optic neuritis were as follows MS (21) polyneuropathy (12) Leber s hereditary optic atrophy (7) meningitis (2) upper respiratory infection (2) and unknown (11) In 25 patients (seven with MS six with Leber s hereditary optic atrophy nine with polyneuro

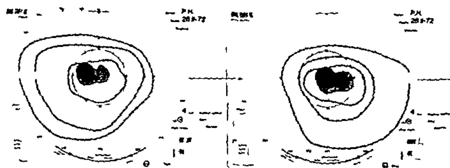


Fig 4

Absolute centrocoecal scotoma in the right and relative centrocoecal scotoma in the left eye in a 25 year old patient with MS Decrease in visual acuities was slow and gradual Visual acuity in the right eye 0 3 and in the left eye 1 0

pathy and three with an unknown cause) decrease in visual acuities was slow and gradual and 11 of these patients had pallor in both optic discs already on admission

On admission visual acuity was still excellent or good in one eye in 14 patients and in both eyes in four patients Both optic discs were oedematous or blurred in 19 patients on admission which in some patients caused a delay in diagnosis

The episode of bilateral optic neuritis was mild in five patients (three with MS one with polyneuropathy and one with an unknown cause) but in 37 patients the disease in the worst phase was associated with poor vision in both eyes In these patients thirteen out of 26 seen 6 months after admission still had poor visual acuity in both eyes

In Fig 3 the visual fields in a patient with polyneuropathy caused by chronic alcoholism can be seen Fig 4 illustrates the visual fields in MS patients with bilateral optic neuritis with a slow and gradual onset

Discussion

The study was an analysis of the ophthalmological symptoms signs and early course of optic neuritis based on the hospital files and interviews of 185 patients These patients represent a typical material in which most patients are young or middle aged with females predominant over males (Bradley & Whitty 1967)

There is no doubt that MS is the commonest cause of optic neuritis (Duke Elder & Scott 1971) In the present study more than half of the patients suffered from MS In 28 % the cause of optic neuritis was unknown It is obvious that at the time of the first attack the cause of optic neuritis often remains unknown With time one patient after the other will be found to suffer from MS This fact was clearly shown in an earlier study (Nikoskelainen & Riekkinen 1974) in which the patients seen during the years 1950–1969 were neurologically re examined Also this was demonstrated in patients seen during 1970–1973 (Table III) because during an attack of optic neuritis only six out of 68 patients were diagnosed as having MS but later during a relatively short observation period (from 6 months to 4 years) there were 22 MS patients

A rather heterogeneous group of patients with optic neuritis consisted of those with metabolic nutritional or infectious causes Optic neuritis in association with subacute combined degeneration nephritis upper respiratory infections the Guillain Barre syndrome and malnutrition caused by alcoholism or by parasites have been reported earlier in the literature (Bjorkenheim 1961 Carroll 1952 Duke Elder & Scott 1971 Foulds 1970 Schlossman & Phillips 1954 and Walsh

& Hoyt 1969) Ischaemic optic neuropathy is a common cause of optic neuritis in elderly persons or in association with vascular disorders (Carroll 1952 Foulds 1970 Schlossman & Phillips 1954) Sinusitis is according to general opinion (Duke Elder & Scott 1971 Walsh & Hoyt 1969) a rare cause of optic neuritis and was suspected as the cause of optic neuritis in only one patient in the present material The optic nerve is surrounded by the same covering as the brain Septa of pia mater penetrate deeply into the optic nerve permitting the direct spread of bacterial or viral diseases (Behrman 1964 Duke Elder & Scott 1971 Miettinen & Wasz Hockert 1960) Two patients in this series had meningitis complicated by bilateral optic neuritis One out of seven patients with Leber s hereditary optic atrophy was a woman The disease affects females about one in seven cases (Meadows 1969)

The commonest first symptom of optic neuritis is a rapid decrease in visual acuity Loss of vision may occur dramatically overnight as described by Benedict (1933) and Meadows (1969) Sometimes the onset may be subacute or slow (Benedict 1933 Duke Elder & Scott 1971 Hierons & Lyle 1959 McAlpin et al 1972) These observations were confirmed in the present study (Tables IV and V)

Pain around and behind the involved eye occurs according to the literature in 20–68 % of the patients and precedes the decrease in visual acuity in 19 % (Bradley & Whitty 1967 Henderson 1956) In the present study the figures were 62 and 16 % respectively Pain is thought to be due to traction of the inflamed nerve by the rectus muscle or related to inflammation or swelling in the adjacent meninges or sheaths of the optic nerve (Henderson 1956 Walsh & Hoyt 1969) One hypothesis is that pain must originate from the pain receptors of the blood vessels in the optic nerve (Behrman 1964) Uhthoff s symptom common in patients with MS (McAlpin et al 1972 Walsh & Hoyt 1969) was present in one fifth of the patients

More than half of the eyes even after 1 month had good or excellent visual acuity This agrees with the observations of Bradley & Whitty (1967) and Earl & Martin (1964) In the series of Bradley & Whitty in 75 % of the cases vision had returned to normal 6 months after the onset In the present study 6 months after the onset 56 % of the eyes had good or excellent visual acuity and the visual field was normal in 45 % of the eyes

The defect in the visual fields mostly affected the central field but there were also eyes (8 %) in which the defect did not involve the fixation area Chamlin (1953) has pointed out that one out of five cases of optic neuritis shows sparing of the fixation area with normal or nearly normal visual acuity

Swelling of the optic disc indicates that the lesion is in the anterior part of the optic nerve In the series of Bradley & Whitty (1967) 41 % of the patients had possible swelling in the optic disc of the involved eye including 17 % with

papilloedema or haemorrhages and 24 % with a blurred or oedematous optic disc A similar figure (43 %) for blurred or oedematous optic discs occurred in the present series Abnormal pupillary function was found in one fourth of the affected eyes Gunn s pupillary sign was a valuable test in optic neuritis as pointed out for example by Wallace (1970)

Bilateral optic neuritis occurred in 30 % of the patients In the series of Bradley & Whitty (1967) 7 % *of the eyes were involved simultaneously* and in 12 % of the eyes the interval between the involvement of the eyes was less than 3 months In patients with bilateral optic neuritis males predominated over females probably because the material included several cases with Leber s hereditary optic atrophy and chronic alcoholism which are more common diseases in males Marshall (1950) also found that males had bilateral attacks more frequently than females There were several patients in whom at least at the onset bilateral optic neuritis involved the one eye only to a minimal extent In one patient the central scotoma in the one eye and a peripheral defect in the other eye the so called junction scotoma (McAlpin et al 1972) would suggest that the pathological process was situated at the termination of the optic nerve Subacute or slow and gradual onset was not an uncommon finding in bilateral optic neuritis Bilateral papilloedema was also found in several patients These types *of optic neuritis present diagnostic problems A central scotoma may* also be an early sign of an expanding lesion of the anterior and middle fossa (Walsh & Hoyt 1969) Some misdiagnosed tumours were found in the original material of optic neuritis" in the present study (Table I)

The present study suggests in accordance with earlier reports that optic neuritis is only rarely an isolated eye disease Therefore every patient with optic neuritis should be neurologically examined Also in patients with unilateral symptoms both visual fields should be carefully studied Patients with optic *neuritis ought to be followed up for several months including repeated* plotting of the visual fields in both eyes This is especially important if the symptoms are atypical if the disease affects both eyes or if there is no tendency to improvement

Acknowledgements

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LATER COURSE AND PROGNOSIS OF OPTIC NEURITIS

BY

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The study was a re examination of 176 patients with optic neuritis The follow up period was for 38 patients 6–12 months for 52 patients 1–5 years and for 86 patients 6–24 years from the initial attack of optic neu ritis In 66 % o the involved eyes visual acuity had again become good or excellent but in 25 % it was poor The visual field was normal in only 38 % in 30 % there was an absolute or relative central defect and in 31 % a paracentral or peripheral defect

Recurrent attacks of optic neuritis occurred in one fourth of the patients and was a common finding in MS patients Nineteen per cent of the eyes had suffered from more than one attack Visual acuity was good or ex cellent in more than half but the visual field was normal in only 29 % of the eyes with more than one attack of optic neuritis

The frequency of bilateral involvement was high at the end of the follow up period 44 % of patients had both eyes involved by optic neuritis In 47 patients the initial attack was bilateral optic neuritis and 34 of these patients had permanently poor vision in both eyes The initial attack was unilateral in 30 patients but the other eye became involved later In 26 % of all patients with bilateral involvement visual acuity was permanently poor in both eyes Bilateral papillitis was a common manifestation in young patients and in this age group the disease had a tendency towards good recovery

Key words optic neuritis – optic neuropathy – optic atrophy – blindness – multiple sclerosis – polyneuropathy – polyneuritis – meningitis – visual fields

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One of the characteristics of optic neuritis is a tendency to spontaneous recovery of vision. The chances of a patient's vision returning to normal or near normal are estimated to be about 50–75 % (Bradley & Whitty 1967, Rawson et al. 1966, Rucker 1956). Since visual acuity may be quite good, the patient often does not notice that a permanent defect has remained in the visual field (Duke-Elder & Scott 1971). Therefore it would be of interest to know the exact frequency of such defects but only a few investigations have been based on the re-examination of patients with optic neuritis including a detailed study of the visual fields (Bradley & Whitty 1967, Hyllested & Møller 1952, Ziegler 1970).

Recurrences are known to be common in optic neuritis and they may attack either the originally involved eye or the other eye. If both eyes are involved simultaneously, the differential diagnosis presents problems. In bilateral optic neuritis there is also a risk of the disease causing permanent blindness. However, there are only few follow-up studies concerning recurrent optic neuritis and bilateral disease (Aschworth 1967, Hierons & Lyle 1959, Lynn 1959, Meadows 1969, Rischbieth 1968).

The present study is a re-examination of patients with optic neuritis in order to clarify the later course of the disease. Special attention has been paid to recurrent optic neuritis and bilateral manifestation of the disease.

Material

The patients in the present study are the same as in the preceding article (Nikoskelainen 1975) except for nine patients (seven with chronic alcoholism, one with possible MS and one with an unknown cause of optic neuritis) who refused to take part in the follow-up. The follow-up period was for 38 patients 6–12 months, for 18 patients 1–2 years, for 31 patients 3–5 years, for 35 patients 6–10 years and for 51 patients more than 10 years. The average follow-up period was 7 years. In patients followed up for less than 1 year the visual fields and visual acuities studied by the author 6 months after admission were normal. Table I shows the patients according to the aetiology and distribution of the lesions.

Methods

The author's re-examination of the patients included taking a careful case history, determination of visual acuities, study of the visual fields and pupils, a

Table I
The patients with optic neuritis divided into groups according to the aetiology and distribution of the disease

Aetiology	Distribution of the disease		Total
	Only one eye involved	Both eyes involved	
Probable or possible multiple sclerosis	49	49	98
Polyneuropathy*	0	5	5
Vascular disease	8	0	8
Leber s hereditary optic atrophy	0	7	7
Infectious disease *	1	4	5
Guillain Barre syndrome	2	0	2
Unknown	39	12	51
Total	99	77	176

* subacute combined degeneration (1) polyneuropathy caused by nephritis (1) poly neuropathy caused by chronic alcoholism (3)
* respiratory infection (2) meningitis (2) sinusitis (1)

biomicroscopical study and ophthalmoscopy as described in detail in the preceding article The intraocular pressure of the eyes was determined if the patient s age exceeded 40 years The colour vision was not systematically studied but tests were *occasionally made with the pseudoisochromatic plates of Bostrom* C G & Kugelberg I and Farnsworth Panel D 15

Definitions

Visual acuity was considered excellent if V $\geq$ 1 25 good if V = 0 8–1 25 fair if V = 0 4–0 7 *and poor if* V $\leq$ *0 3*

Bilateral optic neuritis Both eyes were involved in optic neuritis within an interval of less than 1 month

Bilateral involvement Both eyes had suffered from optic neuritis during the follow up period but not necessarily at the same time

Recurrent optic neuritis The patient had suffered from a new attack of optic neuritis in the same eye or in the other eye within an interval of at least 3 months after the initial attack

Chronic progressive optic neuritis Gradual impairment of vision in MS (McAlpin et al 1972) Patients with repeated attacks of optic neuritis affecting both eyes and those with slowly progressive visual impairment without visual symptoms have been included in this group

Results

Recurrences occurred in 44 (25 %) patients in the MS group the frequency was 40 % (39/98) In addition 26 (15 %) patients reported symptoms that were probably caused by recurrent attacks but there were no objective data available about these episodes It was not an uncommon finding that recurrent optic neuritis affected the same eye because it took place in 47 (19 %) eyes In addition in 12 eyes the findings on re examination suggested a subsequent attack in the same eye In eight eyes there were two subsequent attacks in the originally affected eye in four of these eyes the final visual acuity was good or excellent in two fair and in two poor The author followed up the rate of recovery in eight eyes with repeated attacks and in five of these cases the recovery was as good and as rapid as in the initial attack In 30 (17 %) re examined patients the initial attack had been unilateral but the other eye had become involved later during the follow up period

Visual acuity in the involved eyes on re examination (Table II)

Visual acuity was good or excellent in 66 % of the eyes but in 25 % it was poor In nine eyes visual acuity encompassed only hand movements or less Visual acuity was worse in eyes which had suffered from more than one attack but nevertheless more than half of the eyes with more than one attack had good or excellent final visual acuity

Visual field in the involved eyes on re examination (Table III)

The visual field was normal in only 38 % of the eyes In 30 % of the eyes the defect affected the central field in 13 eyes (7 %) it was associated with periphe

Table II

The visual acuity in 253 involved eyes in 176 patients on re examination

	Visual acuity	Only one attack in the eye		More than one attack in the same eye*		Total	
		No of *eyes*	Per cent	No of *eyes*	Per cent	No of *eyes*	Per cent
Excellent or good	> 1.25	76	39	19	32	95	38
	0.8–1.25	57	30	13	22	70	28
Fair	0.4–0.7	16	8	7	12	23	9
Poor	0.1–0.3	12	6	9	15	21	8
	finger counting or less	33	17	11	19	44	17
	Total	194	100	59	100	253	100

* includes 24 eyes (from 12 MS patients) with chronic progressive optic neuritis In half of these eyes there were one or more subsequent attacks noticed by the patients in the other half of the eyes the later course and re examination suggested repeated attacks in the same eye.

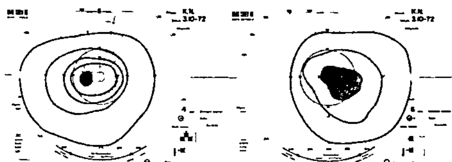


Fig 1

Permanent absolute central scotoma in a 40 year old man 11 years after optic neuritis in the right eye Visual acuity in the right eye finger counting at 2 m in the left eye visual acuity 1.[illegible]5

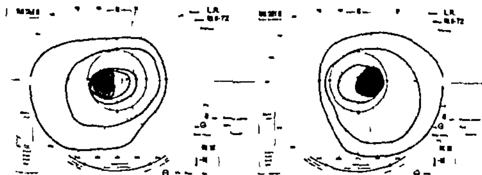


Fig 2

Centrocoecal scotoma in the right and enlarged blind spot in the left visual field in a 27 year old woman 10 years after bilateral optic neuritis Visual acuity in the right eye 0 1 and in the left eye 1 5 All pseudoisochromatic plates missed with both eyes Panel D 15 pathologic in the right but normal in the left eye

ral constriction of the visual field Figs 1–5 show some types of permanent central defects In 31 % of the eyes the defect was paracentral or peripheral Figs 2 and 6 indicate enlarged blind spots after optic neuritis and Figs 7 and 8 visual fields *with nerve fibre bundle defects after optic neuritis Surprisingly* paracentral and peripheral defects were often quite unnoticed by the patients themselves

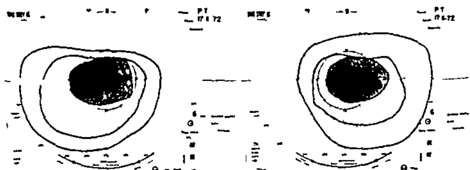


Fig 3

Absolute large centrocoecal scotomas in a 35 year old MS patient 16 years after bilateral optic neuritis Visual acuities in both eyes finger counting at 1 m



Fig 4
Absolute large central defects opening through to the upper periphery in a 36 year old man with Leber s hereditary optic atrophy 2 years after the onset of the disease Visual acuities in both eyes finger counting at 1 m

Peripheral defects occurred in two patients The one patient was a 35 year old woman with MS who after one attack of optic neuritis in the one eye and two attacks in the other eye had made an excellent recovery Later on re examination a homonymous quadrantanopsia was demonstrated in the visual fields The visual acuities were excellent

The other patient was a 44 year old man who came to the hospital because of rapid decrease in visual acuity in the left eye He also complained of pain on movement and

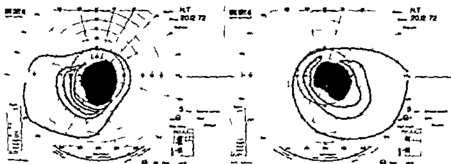


Fig 5
Absolute large centrocoecal scotoma opening through to the upper periphery in a 31 year old man with Leber s hereditary optic atrophy 3 years after the onset of the disease An unusual finding is a small preserved island of vision in the blind area in the right eye Visual acuity in the right eye finger counting at 1 5 m and in the left finger counting at 0 5 m.

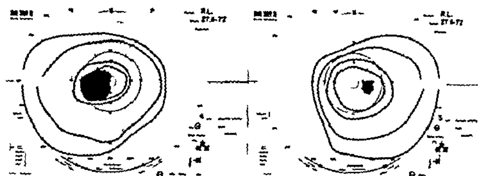


Fig 6

Enlarged blind spot in the left eye in a 49 year old man 18 years after unilateral optic neuritis Visual acuity in the right eye 1 75 and in the left eye 1.5

tenderness on palpation in the left eye but the right eye was completely symptom free The visual acuity encompassed hand movement and the visual field showed a large absolute central scotoma in the left eye, in which the eye fundus was normal The visual acuity in the right eye was excellent but a peripheral relative defect was demonstrated in the visual field The right eye fundus was normal After a follow up period of [illegible] years (on re examination) the left eye was permanently blind showing a pale optic disc The right eye had excellent visual acuity and normal eye fundus The visual fields were unchanged The patient was in good general health but a right sided transient hemiparesis 10 years and a right sided facial palsy 8 years before optic neuritis would suggest a diagnosis of MS



Fig 7

Nerve fibre bundle defects in both visual fields in a 24 year old woman 14 years after bilateral optic neuritis Visual acuity 1 25 in both eyes

Table III

The visual field in 253 involved eyes in 176 patients on re-examination

	Visual field	One attack in the eye		More than one attack in the same eye*		Total	
		No. of eyes	Per cent	No. of eyes	Per cent	No. of eyes	Per cent
	Normal	80	41	17	29	97	38
Paracentral or peripheral defects	Enlarged blind spot and/or nerve fibre bundle defect	52	27	16	27	68	27
	Peripheral defect only	2	1	1	2	3	1
	Paracentral scotoma	7	3	0	0	7	3
Central defect	Relative *central or caecocentral* scotoma	9	5	9	15	18	7
	Absolute central or caecocentral defect	25	13	8	14	33	13
	Absolute defect affecting the central and peripheral field	13	7	5	8	18	7
	Could not be studied because of *amaurosis*	5	2	1	2	6	3
	The visual fields could not be *studied because of extreme* disability of the patient	1	0.5	2	3	3	1
	Total	194	100	59	100	253	100

* includes [illegible] eyes (from [illegible] MS patients) *with chronic progressive optic neuritis*. In one half of these eyes there were one or more subsequent attacks noticed by the patients; in the other half of the eyes the later course and re-examination suggested repeated attacks in the same eye.

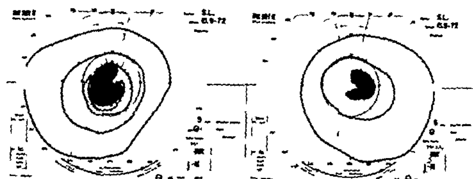


Fig 8

Nerve fibre bundle defects in both visual fields in a 33 year old man 16 years after bilateral optic neuritis Visual acuity 1.5 in both eyes Lanel D 15 normal with both eyes but all pseudoisochromatic plates missed with both eyes

In the eyes with only one attack of optic neuritis the visual field was normal in 41 % On the other hand in eyes with more than one attack only 29 % had a normal visual field on re examination (Table III)

The appearance of the optic disc in the involved eyes on re examination (Table IV)

Sixty per cent of the eyes had slight or total pallor in the optic disc There was often a discrepancy between the appearance of the optic disc and visual acuity In 67 eyes visual acuity was good or excellent but there was pallor in the optic disc The visual field was in better agreement with pallor in the optic disc but in 24 eyes the visual fields were normal although there was pallor in the optic disc Pallor in the optic disc occurred slightly more frequently in eyes after more than one attack

Other findings

Sixty patients (34 %) were of the opinion that there was full visual recovery and 55 (31 %) that there was almost full visual recovery after optic neuritis In most of the patients subjective recovery took place within 2 months after the onset of the disease but in 34 the recovery was slower lasting from 2 to 12 months and in seven patients even longer

Table IV

The appearance of the optic disc in 253 involved eyes in 1 6 re examined patients

Optic disc	One attack in the eye		More than one attack in the same eye*		Total	
	No of eyes	Per cent	No of eyes	Per cent	No of eyes	Per cent
Normal	82	42	18	30	100	40
Temporal pallor	43	22	19	32	62	24
Slight total pallor	27	14	8	14	35	14
Total pallor	42	22	14	24	56	22
Total	194	100	59	100	253	100

includes 24 eyes (from 12 MS patients) with chronic progressive optic neuritis In one half of these eyes there were one or more subsequent attacks noticed by the patient in the other half of the eyes the later course and re examination suggested repeated attacks in the same eye

The colour vision was studied in 23 patients Eight passed tests with both pseudoisochromatic plates and the Farnsworth Panel D 15 with the involved eye Three patients (5 involved eyes) were unable to read the pseudoisochromatic plates but the test with Panel D 15 was normal visual acuities excellent and visual fields normal except for one patient with bilateral involvement who had nerve fibre bundle defects in the visual fields (Fig 8) Slow reaction to direct light was found in 37 (15 %) pupils and 5 (2 %) pupils showed no reaction to direct light stimulus Anisocoria occurred in 13 (7 %) patients

Two patients had pathologically high intraocular pressure and later a diagnosis of chronic glaucoma was established on the basis of the provocative tests In both patients the visual acuities and visual fields were still normal on re examination.

Optic neuritis with bilateral involvement (Table V)

The frequency of bilateral involvement was high being 44 % of re examined patients In forty seven (61 %) out of 77 patients the initial attack was bilateral

Table V
The aetiology and prognosis in 77 patients in whom both eyes were involved in optic neuritis

Aetiology	Initial attack bilateral optic neuritis	Initial attack unilateral but the other eye was involved later	Total
Multiple sclerosis	22 (7)*	27 (4)*	49 (11)*
Polyneuropathy	5 (2)	0	5 (2)
Leber's hereditary optic atrophy	7 (5)	0	7 (5)
Infectious disease	4 (1)	0	4 (1)
Unknown cause	9 (1)	3	12 (1)
Total	47 (16)	30 (4)	77 (20)

* the numbers in brackets denote patients with permanently poor vision ($V \leq 0.3$) in both eyes

optic neuritis. In 30 (39 %) the initial attack was unilateral but the other eye was later involved. Two interesting features were indicated in the patients with MS. Firstly, even in bilateral optic neuritis the most important cause was MS. Secondly, those patients whose other eye was later involved because of a recurrent attack all except three suffered from MS. In 12 MS patients optic neuritis was chronic progressive. Five of these cases had repeated attacks of optic neuritis affecting both eyes. In three patients the chronic progressive course manifested itself as a slowly progressive visual impairment in both eyes without any noticeable episode after unilateral optic neuritis from which a good recovery had been made.

On re-examination 20 (26 %) patients with bilateral involvement had poor visual acuity but 39 (51 %) had good or excellent visual acuity in both eyes. In patients in whom the initial attack was bilateral optic neuritis 16 (34 %) out of 47 had permanently poor visual acuity in both eyes. In five male patients with bilateral papillitis the rate of recovery was unusually slow since 6 months after admission both eyes still had poor vision but later on re-examination the final visual acuities were good or excellent in both eyes. One of these patients

had Leber s hereditary optic atrophy one MS one respiratory infection and two had optic neuritis of unknown cause In all patients who were re examined the frequency of patients with poor vision in both eyes was 11 % (20/176)

Optic neuritis in young patients

Bilateral papillitis was a common manifestation of optic neuritis in children because all eight patients younger than 15 years at the onset of the disease suffered from papillitis and with the exception of one girl the disease involved both eyes simultaneously The onset was acute in all patients

There were 20 patients younger than 20 years on admission The causes of optic neuritis were as follows possible or probable MS (11) meningitis (1) respiratory infection (1) and unknown (7) Table VI shows the ophthalmoscopical findings at the onset and on re examination in 20 patients younger than 20 years on admission Thirteen (65 %) had bilateral optic neuritis and in 27 eyes (82 %) the optic disc was blurred or oedematous during the acute phase On re examination visual acuity was good or excellent in 24 (71 %) eyes Three patients had permanently poor visual acuity in both eyes two of whom were male siblings with MS Fig 3 shows the visual fields of the other sibling on re examination The ophthalmological findings in these siblings in the acute phase suggested Leber s hereditary optic atrophy as the cause of bilateral papillitis but the other neurological symptoms typical of MS appeared later and both patients now suffer from severe disability The third patient with poor vision in both eyes suffered from meningitis complicated by bilateral optic neuritis

In one female patient the final visual acuity was poor in the one eye but excellent in the other eye Her visual fields on re examination are shown in Fig 2 Figs 7 and 8 present the visual fields of one female and one male patient in whom both optic discs were pale but visual acuities normal on re examination The male patient has a brother who was also included in the present material because of a similar case history Both of these patients suffer from possible MS

Ophthalmological prognosis according to the aetiology

The most common cause both in unilateral and bilateral involvement of optic neuritis was MS It was the cause of permanently poor vision in both eyes in 11 out of 20 patients On re examination four out of eight patients with ischaemic optic neuropathy caused by vascular disease had made a good or excellent re

Table VI

The visual acuity visual field and appearance of the optic disc in the involved eye of 20 patients with optic neuritis and younger than 20 years*

Includes 13 patients with bilateral and seven with unilateral optic neuritis The follow up period was from 3 to 24 years

Visual acuity	At onset of optic neuritis		On re examination	
	No of eyes	Per cent	No of eyes	Per cent
> 1.25	3	9	22	65
0 8–1 25	2	6	2	6
0 4–0 7	6	18	3	9
0 1–0 3	6	18	1	3
Finger counting or less	16	49	6	17
Total	33	100	34**	100
Visual field				
Could not be studied because of amaurosis	1	3	0	0
Absolute central or caeco central defect +/– peripheral defect	13	40	6	18
Relative central scotoma	2	6	1	3
Paracentral scotoma	1	3	1	3
Enlarged blind spot +/– nerve fibre bundle defect	4	12	18	53
Peripheral defect only	1	3	0	0
Unreliable	6***	18	0	0
Not studied	4***	12	0	0
Normal	1	3	8	23
Total	33	100	34	100
The appearance of the optic disc				
Normal	5	15	22	65
Blurred and/or hypaeremic	10	30	0	0
Papilloedema	17	52	0	0
Temporal pallor	1	3	0	0
Slight total pallor	0	0	5	15
Total pallor	0	0	7	20
Total	33	100	34	100

* the age range from 6 to 19 years

** the number of eyes were increased by one because of a recurrent optic neuritis in the other eye after a 6 months interval

*** the patients did not co operate because of young age

covery but in others the prognosis was poor Only one patient with polyneuropathy had good visual acuities on re examination The prognosis was also poor in patients with Leber s hereditary optic atrophy because five had permanently poor vision Two patients (female and male siblings) had a slow recovery leading to good visual acuities The prognosis was good in two patients with respiratory infection and one with meningitis but in one patient with meningitis it caused permanent blindness In a patient with sinusitis as the possible cause of unilateral papillitis visual acuity in the involved eye on re examination was fair

Discussion

The present study showed that one fourth of the patients suffered from recurrent attacks of optic neuritis The frequency of recurrences was even higher (34 %) in an earlier study (Nikoskelainen & Riekkinen 1974) in which 116 patients from the present material were neurologically and ophthalmologically re examined at least 3 years after the initial attack of optic neuritis In that study a recurrent attack strongly suggested a diagnosis of MS Although in the present study the initial attack was unilateral in 73 % (129/176) 44 % of the patients had both eyes involved by optic neuritis on re examination after 7 years on the average

In the preceding article 56 % of the eyes had good or excellent visual acuity but 37 % had poor visual acuity 6 months after admission In the present study 66 % of 253 eyes had good or excellent visual acuity after optic neuritis but only 25 % had poor visual acuity Thus even the later course of vision is good in optic neuritis although there may be recurrences The relatively good prognosis of visual acuity confirms the observations made in earlier studies (Bradley & Whitty 1967 Rawson et al 1966 Rucker 1956) The frequency of patients with poor vision in both eyes was 11 % of all re examined patients This figure was higher than in Marshall s (1950) series (6 5 %) but more than half of the patients in his study were seen only once

According to the literature recurrences in an eye that was previously affected occur in 10–15 % (Rucker 1956) In the present study the figure was slightly higher being 19 % Probably the frequency was in fact even higher because in some patients the symptoms and findings suggested a recurrent attack in the same eye Visual acuity in eyes with more than one attack was not much worse than in eyes with only one attack This is in agreement with the study made by Rischbieth (1968) but Lynn (1959) reported a worse prognosis in vision

Permanent central or paracentral defects in the visual fields occur in 17–43 % according to the literature (Bradley & Whitty 1967 Hyllested & Moller 1952 Ziegler 1970) In the present study the frequency of pathological findings in the visual fields after optic neuritis was higher since the visual field was normal in only 38 % of the involved eyes This indicates that visual acuity alone was an inadequate record for recovery in optic neuritis Common findings were para central defects after optic neuritis often unnoticed by the patients Similar defects after optic neuritis have been reported for example by Hyllested & Moller (1952) and Schlossman & Phillips (1954) A plaque of demyelination may also be situated in the optic chiasma optic tract or optic radiation (Walsh & Hoyt 1969) In the present study one MS patient with homonymous quadrant anopsia probably presented a case where plaque was situated behind the chiasma in the optic pathway In one patient the central scotoma in the left visual field and the peripheral defect in the right visual field the so called junction scotoma suggested that the process was situated at the termination of the left optic nerve near the chiasma (McAlpin et al 1972)

A recurrent attack in the same eye had a tendency to induce more defects in the visual field Schlossman & Phillips (1954) have suggested that many re currences are missed because some of the repeated attacks only partially involve the fixation area but nevertheless leave a certain amount of residual damage Autopsy studies have also shown that pathological involvement in the optic nerve is more extensive than one would except from the visual acuity alone (Gartner 1953 Schlossman & Phillips 1954)

There was often discrepancy between visual acuity and the appearance of the optic disc Sometimes there was also discrepancy between the appearance of the optic disc and the final visual field Estimation of the appearance of the optic disc was sometimes difficult as pointed out earlier by Lynn (1959) Carroll (1956) has pointed out that the colour of the optic disc is often an unreliable indication of the nerve s conductivity power but if there has been a definite change in the colour and if the visual field appears to have been permanently impaired a clinical diagnosis of optic atrophy is probably justified

Both eyes were involved by optic neuritis in 44 % In an earlier study (Nikos kelainen & Riekkinen 1974) with a longer follow up period 45 % of the patients had both eyes affected In the series of Schlossman & Phillips (1954) the fre quency of bilateral involvement was also 45 % (25 out of 55 patients) other authors have reported lower frequencies e g 29 % (Bradley & Whitty 1967) Bilateral involvement carried a serious prognosis because 26 % of the patients had permanently poor vision in both eyes In patients with bilateral optic neuri tis as the initial manifestation of the disease the prognosis was even worse since 34 % had bilateral poor vision on re examination

In some patients with bilateral papillitis recovery was unusually slow but led to good visual acuities in both eyes Similar cases have been reported earlier (Carroll 1952 Scott 1961 Walsh & Hoyt 1969) Spontaneous recovery in Leber s hereditary optic atrophy have been described for example by Brunette & Bernier (1970)

Bilateral papillitis was a common manifestation of optic neuritis at a young age The recovery of visual acuities was good in the vast majority of patients Results are in accordance with earlier studies concerning optic neuritis in young patients (Hierons & Lyle 1959 Kennedy & Carroll 1960 Meadows 1969)

In twelve MS patients optic neuritis was chronic progressive Repeated attacks of optic neuritis affecting both eyes as well as slowly progressive visual impairment without visual symptoms may occur in optic neuritis (Aschworth 1967 Hierons & Lyle 1959 Kahana et al 1973) In some patients with MS the demyelinating disease is insidiously progressive (Broman 1970) About 50 % of MS patients later move into a phase of steady progress (Andersen et al 1974) Apparently the course of optic neuritis may reflect the course of demyelinating disease in MS patients The ophthalmological prognosis of optic neuritis in patients with MS has been studied in an earlier article (Nikoskelainen & Riekkinen 1974)

In conclusion studies concerning only the initial attack of optic neuritis give an incomplete picture of the clinical course of the disease since with a long enough follow up period almost half (44–45 %) of the patients have both eyes involved in optic neuritis Although there are recurrences the prognosis of visual acuity is relatively good but permanent defects in the visual fields are common Bilateral involvement of optic neuritis has a more serious prognosis especially if both eyes are involved simultaneously More than half of the patients with optic neuritis suffer from MS A recurrent attack of optic neuritis strongly suggests a diagnosis of MS

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74th Meeting of the German Ophthalmological Society

The Annual Meeting of the German Ophthalmological Society will be held from September 11 through 24 1975 at the Haus der Technik Essen Germany The main subject is *Peripheral Retina* Simultaneous translation in German English and French For additional information write to Prof Meyer Schwickerath D 43 Essen Universitats Augenklinik President of the Society or Prof Jaeger permanent Secretary of the Society

17 Tagung der Österreichischen Ophthalmologischen Gesellschaft

Innsbruck 29–31 Mai 1975 Vorlesung gehalten von P Y Evans Fluorezensangiographie Freie Vortrage *Sekretariat* Augenklinik der Universitat (Prof Dr K Heinz) Anichstrasse 35 A 6020 Innsbruck Österreich

56th Ophthalmological Meeting of the Italian Society

The meeting of the Italian Society will be held in Rome (Hotel Hilton) from 28th to 30th November 1975 and the main subject concerns with The visual field Free lectures and scientific films are admitted Official language Italian – English For informations Dr I Esente – Corso Italia n 2 50123 Florence (Italy)

XXII Meeting of Nordic Ophthalmologists Lund Sweden June 4 to 7 1975

The twenty second Meeting of Nordic Ophthalmologists will take place at the University of Lund Sweden June 4 to 7 1975 The main subject of the meeting is Recent clinical methods of examination Official languages Scandinavian Regarding informations please write to Professor Erik Palm Dept of Ophthalmology University of Lund S 221 85 Lund Sweden

The Department of Experimental Ophthalmology
(Head Prof C E T Krakau)
University Eye Clinic Lund Sweden

AN AUTOMATIC STATIC PERIMETER
DESIGN AND PILOT STUDY

BY

A HEIJL and C E T KRAKAU

An instrument for automatic static perimetry at 14 points across a meridian was constructed and used in a pilot study The machine was controlled by a computer and the subjects responded to the light stimuli by pressing one of two push buttons Most people investigated readily understood the rules at testing Blunders made by the subject were however so common, that allowance had to be made in the test programme for a number of mistakes The test stimuli should be exposed in random order

When the zone of uncertain seeing is wide – this occurs especially in untrained subjects – a simple test logic gives a fairly large variation An improvement in reproducibility is then obtained by applying a more complicated logic though at the cost of an increased duration of the test session

Key words visual field – perimetry – automatic – perimeter – computer – static perimetry

It is generally acknowledged among clinicians that the recordings of routine visual fields are often of inferior quality even in places where the importance of these investigations is fully understood A good perimeter is a prerequisite but the ability of the perimetrist is of paramount importance We have to agree with Goldmann (1969) when he says *ich möchte betonen dass Perimetrie besonders kinetische Perimetrie eine Kunst ist*

Received January 1[illegible]

When plotting a visual field we aim at minimizing the possible deviation from a presumed true visual field and getting the least possible dispersion in repeated recordings There are errors in the recording due to the subject s shortcomings – tiredness difficulties in concentrating on the test boredom But the same imperfections afflict the perimetrist The variation introduced by the examiner is by no means negligible (Niesel 1970) By replacing the perimetrist with some automatic device one of these two sources of error could be reduced

In the past several semi automatic devices for testing the visual field have been presented like the Globuc device (Buchanan & Gloster 1965) the Friedmann visual field analyzer (Friedmann 1966) and the Fincham Sutcliffe screening scotometer (Sutcliffe 1963) Recent development in the field of electronics makes it possible to solve some of the problems involved in the construction of a fully automatic perimetric device A number of commercially available automatic devices (Ocutron Automatic Electronic Perimeter Biotronics Auto Field I) and a new technique for objective plotting of visual fields (Jernigan 1974) have also been introduced The performances made possible by means of thes machines are still on a fairly simple level usually only one stimulus intensity is used in every tested point A more ambitious approach has been taken by a group in Bern who have discussed some fundamental points of the theoretical side of visual field determination (Fankhauser et al 1972 Koch et al 1972) and also presented a device for recording based on a minicomputer (Spahr 1973)

There are no doubt several basic questions unsolved concerning the behaviour of the patient in an impersonal automatic testing system Can we expect the average patient to work for himself with the machine without continuous encouragement from the perimetrist? Will he show decreasing attention to the task and give unreliable answers after some time? Are there some modes of test object presentation less inclined to provoke erroneous answers than others?

The aim of this paper has been to construct a not too complex apparatus capable of a completely automatic testing of the visual field at a number of points along any meridian and to essay various testing conditions with a view to optimizing the test logic

Material

Eight experimentees in the range 22 to 62 years of age were used All the eyes tested were normal The persons tested were corrected for ametropia and for near vision as is customary in conventional perimetry Five of the subjects had no previous training whatsoever in visual experiments They were completely

unacquainted with the apparatus when the measurements started and only given a short instruction on how to respond

A fairly large number of other persons were also tested but only on a few occasions each These results are therefore not suitable for the present analysis and these measurements have not been dealt with in this paper

Methods

Perimeter set up

On the arc of a Maggiore perimeter a piece of board 31 × 43 cm painted matt white was mounted This was evenly illuminated The luminance was 30 cd/m Centrally a red light emitting diode (LED) was placed as a fixation light As test lights LEDs of yellow emission (Monsanto MV 5392) were used These were mounted in special holders on the back of the board The light was projected through small plexiglass light leaders These were fixed in holes 2 mm in diameter in the board Fourteen diodes placed along a meridian at 2.5° 5° 10° 13 3° 16 7° 20° and 25° from the fixation light were used. This is in agreement with the opinion of Blum et al (1959) who claim that the peripheral parts of the visual field are of minor interest The whole board could be rotated so that any meridian could be investigated

One LED was placed at the turnable, mirror containing tube above the patient s head. The light from that diode was projected on to the screen by means of a simple lens The projected light spot could be freely moved just like the original test light of the Maggiore perimeter It was adjusted to fall in the blind spot area In front of the eye to be tested was a holder for correction glasses

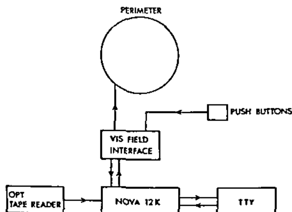


Fig 1
Block diagram

In all 15 test lights were used the first one being reserved for the blind spot This light was fixed at a high intensity level The other 14 lights could take any one of 15 intensity levels The ratio between the intensities of two consecutive levels was $\sqrt{2}$

Computer

The visual field device was controlled by a computer Nova 1200 (Data General) with 12 k 16 bits word memory in combination with an optical paper tape reader and a teletype (Fig 1) Via a specially designed interface the visual field device was connected to the General Purpose Interface of the computer The programme language Extended Basic was used Input and output orders were given directly from the Basic programme by means of Call statements

The *output* word from the computer was either a reset signal (see below) or a word which told which of the lights to be lit (four bits) plus the intensity of the light (four bits) As soon as an order to activate one of the test lights was given by the computer the visual field device translated the output word (from the computer) into a current of 0 5 sec duration and at specific level to one of the test lights The patient responded to the test stimulus by pressing one of two buttons (left/right) or he did not respond at all Meanwhile the data programme rested in a short loop which was broken when a button was pressed or the waiting time after the stimulus (2 0 sec) was over The visual field device sent a message as an *input* word to the computer telling whether the patient had responded and how The result was stored by the computer a reset signal went to the visual field device and wiped out the last interpretation and a new test light at a new intensity level was chosen and exposed 0 5 sec later At the end of a test the results were immediately written out numerically and as a tabulated curve by the teletype

Test Logics and Results

Statistical model

When constructing the different types of test logic we were guided by a statistical model which we have a priori found reasonable

A There is at each tested point a faintest intensity at which an object is always seen except in totally blind areas The probability of seeing this object is then 1 (Fig 2 p = 1)

B There is at each point a strongest object which is never perceived The probability of seeing is 0 (Fig 2 p 0)

C Between these objects there are intermediate objects seen with a probability greater than 0 and less than 1

D The threshold range between the objects always seen and never seen for untrained subjects comprises several steps of the size ($\sqrt{2}$) we have chosen.

E We presume that the testing time is short enough to permit us to consider the probabilities of perceiving an object to be stationary

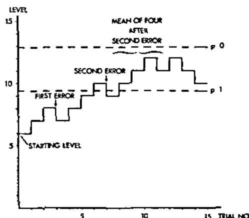


Fig 2

Example of test procedure at one single test point Stimulus level number increases i e stimulus intensity is reduced with every correct interpretation and decreases with every false answer or when no answer is given

The test was performed according to the following rules a correct interpretation changed the level of the test object by one step to the next higher level number (weaker stimulus) a false answer or no answer at all made the level one step lower (stronger stimulus) (A more extensive discussion of this model is given by Krakau (1969))

Presuming no blunders are made by the experimentee the process will finally be caught between the levels where p = 1 and p = 0 (Fig 2) It can be described as a Markov process with reflecting barriers and its behaviour is determined by the set of transition probabilities (They were earlier called probabilities of seeing) The transition probabilities can be estimated by counting the number of transitions (n) from a level (i) to a more difficult one (i + 1) and those to an easier one (i – 1)

$$p_{\quad i} = \frac{n_{\quad i}}{n_{\quad i} + n_{\quad i}}$$

This model is an idealization Thus for instance the assumption of stationarity has a limited validity

The process is brought to an end by some arbitrary rule such as when the second error of interpretation appears the process is finished The probability that the process ends at some specific level is given by a set of end probabilities which can be calculated from the rule and the set of transition probabilities

Reference programme

A number of practical questions have to be solved before we can approach the problem of finding an economical test logic Therefore a main programme serving as a reference for other test programmes was constructed It had the following specifications

a) The stimuli appeared in a random fashion generated by a random generator in the computer

b) At the beginning of a test session the starting levels of the different lights were those illustrated in Fig 3 A higher level number here means a weaker stimulus Thus the central stimuli had a lower intensity than the peripheral ones

c) If the experimentee responded adequately to a given stimulus e g pressed the left button if one of the test lights to the left of the fixation mark lit up the intensity of that same lamp was reduced by $1/\sqrt{2}$ the next time it appeared On the other hand if he did not respond at all or pressed the wrong button that same stimulus had an intensity which was $\sqrt{2}$ higher at the next presentation

d) If the patient had seen a stimulus in that same position before the stimulus initially being supra threshold and later did not interpret a given stimulus correctly at that point this point was not tested any more If the stimulus initially was not seen the stimulus intensity was increased at every presentation until the patient indicated that he saw the light or the maximum value of the visual field device was reached The last correctly interpreted level was taken as threshold level

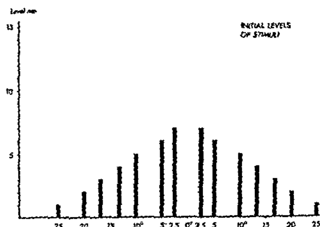


Fig 3
Intensity levels of test stimuli at beginning of tests

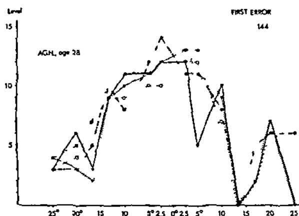


Fig 4
Five curves across the horizontal meridian of the right eye
First error logic = reference programme

Normally the stimulus at the beginning of the test being visible the stimulus intensity would thus decrease in a step wise fashion until the patient did not see the light any more or did not respond correctly to the stimulus (Fig 2)

The diagrams marked first error (Figs 4 and 6) show typical static profiles obtained in using this reference programme All curves are pro' les in the

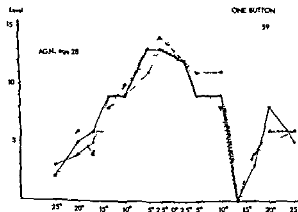


Fig 5
One button logic (see ad 3) Same subject as Fig 4

Table I

Subject Age of subject Test programme	AGH1) 28 s d	 Time	AMP1) 23 s d	 Time	EE1) 62 s d	 Time	FSS1) 22 s d	 Time	UR1) 57 s d	 Time	WT 31 s d	 Time	AH 25 s d	 Time	PW 32 s d	 Time
First error	1 47	144	1 23	162	–	–	1 44	156	1 07	163	1 61	176	0 95 3)	144	0 75	168
One button	0 99	159	0 88 3)	158	–	–	–	–	–	–	1 30	169	0 82	148	–	–
Non random	1 35	153	–	–	–	–	–	–	–	–	–	–	0 95	160	–	–
Second error	1 13	219	–	–	1 19	200	1 02	224	1 01	218	–	–	0 79	205	1 13	276
Mean of four	1 01	391	1 13	335	0 90	376	0 83	324	0 73	334	–	–	0 72 3)	309	0 64	329

s d Denotes the mean of the standard deviation at all points tested except the points in the blind spot area The same test programme was repeated five times with each person except 2) where 10 measurements were made and 3) which is based on three measurements Mean testing is given in sec 1) denotes untrained experimentee

horizontal meridian of the right eye* Degrees of eccentricity from the fixation mark are represented on the abscissa Mean testing time in sec is given in the upper right corners Test persons were completely untrained Standard deviations of more tests using this programme are shown in Table I first error In all our experiments we found no significant difference in variation between central and more peripheral test points

Design problems

The set up described has great flexibility within its mechanical limits making it easy to vary the test conditions Thus it ought to be possible by introducing certain control devices in the logic to improve the reproducibility of the results The possible gain from such devices must however be balanced against the extra time they inevitably require If the testing process is made too complicated and time consuming the patient may become tired and careless thus counteracting the purpose of the control mechanisms In our attempts to optimize the testing procedure we are faced with several fundamental questions

1 How can we know whether the patient maintains fixation or not?

2 Is it an advantage to present the test points at random instead of taking them in sequence?

3 Is it an advantage to use two answering alternatives for the patient (left/right) instead of only one (light is seen)?

4 Can we obtain better results by taking into account some blunders made by the patient?

5 Are there other practical methods of getting more reproducible results say by taking an average of the latest trials?

6 Do the patient s test results deteriorate if the test session is prolonged? If so for how long can a testing session go on in practice?

ad 1 Fixation There are methods for a direct control of fixation e g by using the corneal reflex from an infrared light source Such a device however may be difficult to use in combination with correction glasses and since correction for near vision is a necessity in most patients we have used another method for fixation checking A light spot was now and then projected at the blind spot area This stimulus had a comparatively high fixed luminosity If the patient indicated having seen it by pressing a button he was not fixating correctly His answer was stored by the computer At the end of the test we got a number

* The systematic deviations from what is theoretically expected present in these curves and the other curves of this paper can be explained by irregularities of mounting between different test points

revealing how many times the patient had reacted to the blind spot light This figure is an indication of the patient s fixation reliability Test sessions in which the patient gave more than four such erroneous answers were discarded Usually a cooperative patient gave between 0 and 2 such answers in one test session

ad 2 Order of objects A primary question in choosing test logic concerns the order in which the objects are shown Two types of programmes were compared 1) Starting from the most central objects and ending at the most peripheral ones each point was investigated to its final value (see appearance of the first error) before the next one was tested 2) The points were tested in random order (reference programme) Let A B C be the objects and the intensity level given as a subscript (B_5 being the fifth intensity level at the test point B) Then programme 1 means a sequence A_1 A A_n B_1 B B etc and programme 2 a sequence like E_1 B_1 C_1 E A_1 E_3 C Not unexpectedly there was a tendency towards side glancing when the objects were taken in sequence This was revealed by the greater number of blind spot answers in the latter case The mean number of blind spot answers per test session for subjects AH and AGH respectively was thus found to be 0 8 and 1 83 with stimuli randomized and 1 8 and 5 25 when the stimuli were exposed in sequence (Test curves with more than four answers on blind spot stimulus presentation were not discarded in this calculation) This tendency to side glancing was also reported by the test subjects Therefore we have preferred the random order system

ad 3 One or two buttons In the ideal patient a useful level value between the barriers for probability of seeing 0 and 1 (Fig 2) would be obtained with a one button logic But since each pressing of a button is reckoned as a correct interpretation a mischievous overambitious or otherwise unideal patient may reach completely meaningless results A great number of answers on blind spot stimulus presentations would disclose such a patient

If we use a system of two buttons (corresponding to object seen to the right or to the left) one may suggest (Barany 1946) the following relation between the probability of a correct choice (p) of pure guessing (p_g) and of true perception (p_v) $p = p + p_g (1 \; p_v)$ In a forced choice situation the patient always guesses even if he does not see the object with certainty ($p_g = 0\ 5$) and thus $0\ 5 \leqq p \leqq 1\ 0$ Even in the worst case with a patient always gambling when not seeing the probability of reaching *a level far above the threshold zone* is rapidly falling with the distance from the threshold

A few test series were made using only one push button but otherwise having a logic identical to that of the reference programme The results are given in Table I one button and Fig 5 (to be compared with Fig 4) They show that in all four subjects used the dispersion is somewhat smaller when only one

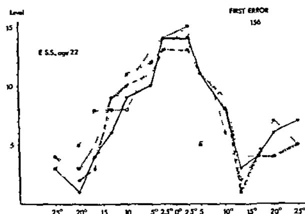


Fig 6
First error logic

button is used This indicates that the subjects used are not much addicted to gambling It also seems likely that the two choice system puts an increased intellectual burden on to the subject which may influence the results unfavourably instead of being a desirable control mechanism

ad 4 Blunders In the reference programme we let the stimulus decrease in a step wise fashion as long as the patient answered correctly and the first mistake

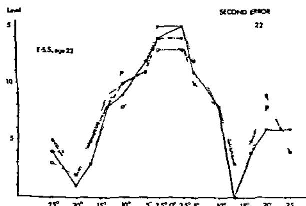


Fig
Second error logic (see ad 4) Same subject as Fig 6

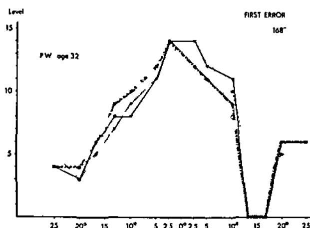


Fig 10
First error logic Trained experimentee

one step lower than the end level number for a one and a two error logic (first error and second error logic described above) are shown (Fig 9 A and B resp) Our experiments are in agreement with our model simulations From Fig 8 (cf Figs 6 and 7) and Table I it is clear that the dispersion of the levels was smaller when using a mean of four logic than in the first and second error series

Although it is possible for a trained experimentee to achieve rather comparable results when using a simple logic and a more sophisticated logic (Figs 10

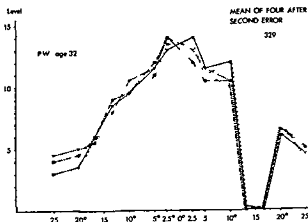


Fig 11
Mean of four logic Trained experimentee Same subject as Fig 10

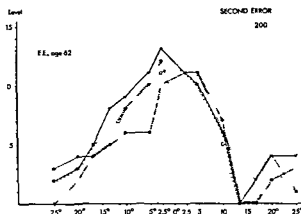


Fig 1?
Second error logic In patient

and 11 respectively and Table I subject AH) an untrained subject e g a patient will produce more consistent results when using the mean of four logic (Figs 12 and 13)

ad 6 Time aspect The time needed for an examination is crucial In discussing the transition probabilities above stationarity was implied but this can be true only for a limited period That this is a fact is clear from our experience with

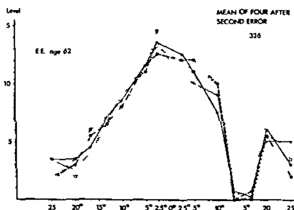


Fig 13
Mean of four logic In patient same subject as Fig 12

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INTRAVITREAL INJECTION OF VANCOMYCIN IN EXPERIMENTAL STAPHYLOCOCCAL ENDOPHTHALMITIS

BY

PAUL HOMER GHOLAM A PEYMAN JEFF KOZIOL
and DONALD SANDERS

Toxicity clearance and therapeutic effectiveness of intravitreal vancomycin hydrochloride injection in experimentally induced staphylococcal endophthalmitis were evaluated Vancomycin was found to be nontoxic in a single 1 mg/0 1 ml intravitreal dose Therapeutic levels of vancomycin were present in the vitreous for over 72 hours and in the aqueous during a period from 6 to 48 hours after injection Injection of a methicillin resistant *Staphylococcus aureus* produced a panophthalmitis in our systemically treated controls whereas in the rabbits treated by intraocular injection the course of the infection was significantly altered

Key words vancomycin – intravitreal injection – endophthalmitis – antibiotics

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Received December ? 1974

Staphylococcus aureus has been estimated to be responsible for up to half of postsurgical bacterial endophthalmitis (Allen et al 1964) The infection progresses rapidly and even with prompt treatment useful vision is lost in the majority of cases Despite treatment with corticosteroids and antibiotics the prognosis is poor due partially to *poor penetration into the vitreous compartment* by antibiotics administered systemically subconjunctivally or topically

The sources of staphylococcus are many A direct relationship between staphylococcus on the lid margin and those recovered from postoperative endophthalmitis has been shown through phage typing of the strains (Locatcher Khorazo *et al 1960) Other* possible sources of staphylococcal infection are direct extension from a periorbital abscess or the surgeon s nasal flora

There are two major problems in treating staphylococcal endophthalmitis First conventional modes of therapy i e subconjunctival topical and systemic administration of drugs effective against staphylococcus do not achieve adequate levels in the vitreous (Deyer 1962 Furguiele 1960 1964 1967 and Leopold 1945 1946) The second problem involves rapidly emerging strains of staphylococcus *resistant to previously effective drugs Methicillin once the drug of choice* against penicillinase producing staphylococcus is no longer as effective Staphylococci insensitive to this agent have been isolated from patients in Europe Africa and the United States the frequency varying from 0 3 % to 4 % Such strains are usually resistant to penicillin G streptomycin sulfate kanamycin *sulfate chloramphenicol and the tetracyclines and frequently to lincomycin* hydrochloride monohydrate and the cephalosporin derivatives (Goodman et al 1970)

To solve the first problem Peyman (1974) May (1974) and Daily (1973) have had marked success at achieving adequate intraocular drug levels by intravitreal injection of various drugs

The second problem of drug resistance can be solved only by using newer and more potent drugs against staphylococci Vancomycin hydrochloride which we decided to use in this investigation is relatively new The primary action of vancomycin is against *S aureus* inhibiting most staphylococcal cultures with under 6 μg of drug per ml A study at Jefferson University Hospital showed that vancomycin was the only drug of 11 tested effective against every staphylococcal culture tested over a 5 year period (Wise 1973)

Vancomycin is not chemically related to any of the presently used antimicrobial agents and cross resistance with other antimicrobial agents has not been demonstrated Vancomycin inhibits bacterial cell wall synthesis probably by interference with glycopeptide polymerization (Goodman et al 1970)

We administered vancomycin intravitreally as effective intraocular levels of the drug can not be achieved by the usual three modes of treatment (Pryor et al 1962)

Materials and Methods

Commercially available vancomycin (Vancocin Hydrochloride 500 mg in a 10 ml rubber stoppered ampule) was used in all parts of this study Distilled water was added to each ampule to dilute the amount of vancomycin to the required concentration

Toxicity

Ten albino rabbits each weighing 2 to 3 kg were anesthetized with intravenous pentobarbital sodium and given 0 12 mg of intramuscular atropine to control excessive secretions Two drops of 1 % cyclopentolate hydrochloride and two drops of 0 5 % proparacaine hydrochloride were instilled into the conjunctival sac on both eyes of each rabbit for pupillary dilation and topical anesthesia The superior and inferior rectus muscles were grasped with toothed forceps for stabilization of the eyeball A 27 gauge needle attached to a tuberculin syringe was inserted into the vitreous at the temporal pars plana The needle was directed posteriorly into the vitreous to avoid contact with the lens When the bevel of

Table I
Toxicity of vancomycin

Concentration of drug injected mg	No of eyes injected
0 25	1
0 5	1
1 0	2
2 0	2
3 0	2
4 0	2
5 0	3
10 0	2
200 0	1
500 0	2
	Total 18

One rabbit in each group injected in the right eye only The left eyes serving as controls each received 0 1 ml of normal saline

the needle could be seen centrally in the vitreous anterior paracentesis with a 25 gauge needle was done and 0 1 ml of vancomycin hydrochloride was injected We varied the concentration of vancomycin injected from 0 25 mg to 500 mg per 0 1 ml of distilled water (Table I) Two of the rabbits were injected in the right eye only one with 5 mg and one with 10 mg the left eyes injected with 0 1 ml of normal saline served as controls After injection the eyes were dilated daily with 1 % cyclopentolate and observed ophthalmoscopically for changes At 2 weeks the animals were killed The eyes were enucleated and examined histologically

Clearance

Eight albino rabbits each weighing 2 to 3 kg were anesthetized and dilated as previously described Each rabbit received an 0 1 ml injection of 1 mg of vanco mycin hydrochloride in both eyes The rate of clearance from the aqueous and vitreous was then determined At 0 1 2 4 6 24 48 and 72 hours the rabbits were killed The eyes were enucleated and immediately frozen in liquid nitrogen The vitreous and aqueous were then dissected from the eye The samples were put in an antibiotic medium with a pH of 8 0 *Sarcina lutea* was used as the indicator organism With this procedure we were able to detect the presence of vancomycin to 9 μg/ml Below that there were no zones of inhibition

Treatment of experimentally induced endophthalmitis

Ten albino rabbits anesthetized as previously described received intravitreal injections of 10 000 methicillin resistant *S aureus* organisms into each eye The 10 rabbits were then divided into two equal groups one group received therapy at 8 hours and the other group at 12 hours Treatment consisted of a single intravitreal injection of 1 mg of vancomycin into the right eye All 10 rabbits received 25 mg/kg intravenously and the left eye served as a control The rabbits were observed hourly for the first 12 hours and then once a day for 2 weeks At the end of this time the rabbits were killed and the eyes were studied histologically

Results

Toxicity

Gross observation The cornea lens and media appeared clear after intravitreal administration of less than 5 mg of vancomycin At dosages of 5 mg or greater

a whitish reaction was noted in the vitreous that lasted 2 weeks At that time electroretinograms were done on the rabbits eyes and compared to ERGs done prior to injection of vancomycin The ERGs on the rabbits given 1 mg of vancomycin or less were interpreted as normal

Histologic evaluation The eyes injected with 1 mg or less showed no toxic changes histologically (Fig 1) At 2 to 5 mg there was evidence of localized toxicity to the retina (Fig 2) The extent of the damage to the retina increased in proportion to the dose of vancomycin injected At dosages greater than 10 mg there was total retinal destruction The two control eyes were completely normal histologically From these results we determined that a safe intravitreal dose of vancomycin is 1 mg

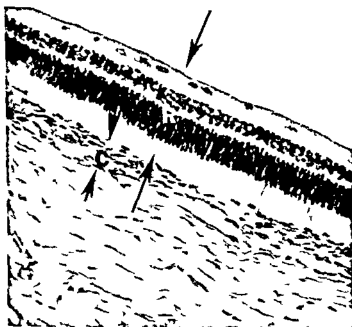


Fig 1

Photomicrograph of eye 2 weeks after intravitreal injection of 1 mg of vancomycin The histologic appearance of the retina (R) is completely normal C indicates choroid (hematoxylin and eosin × 250)

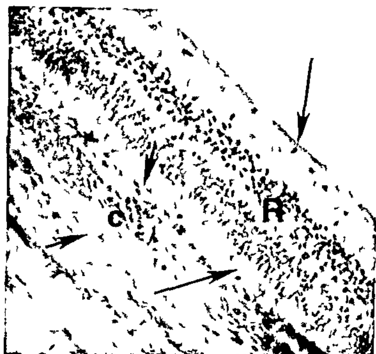


Fig 2

Photomicrograph of retina (R) *and choroid* (C) *showing degeneration of photoreceptor* outer segments Eye received 5 mg intravitreally (hematoxylin and eosin x 950)

Table II

Clearance of vancomycin from vitreous and aqueous

Hours	Level in vitreous μg	Level in aqueous μg
0	500	No zone
1	550	No zone
2	475	No zone
4	400	No zone
6	260	37
24	300	25
48	190	9
72	130	No zone

Clearance

The results of the clearance study can be seen in Table II Intravitreal injection gave immediate therapeutic levels in the vitreous (Fig 3) The peak intravitreal level of 550 μg was over 50 times the needed inhibitory concentration for almost any staphylococcal infection encountered clinically The vitreous level was still high as long as 72 hours after injection Aqueous levels were detectable 6 hours after injection and stayed significantly elevated for at least 42 more hours There were probably levels in the therapeutic range 48 hours after injection but our assay was not sensitive enough to detect antibiotic levels under 9 μg/ml

Treatment of experimentally induced endophthalmitis

The first evidence of infection occurred 4 to 8 hours after injection when pin point opacities surrounded by cloudy media were first seen in the posterior

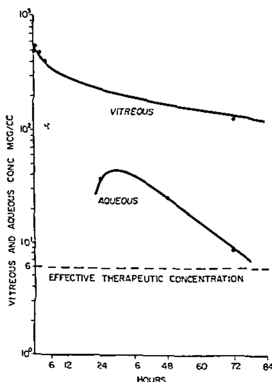


Fig 3
Results of clearance study from aqueous and vitreous after intravitreal injection of 1 mg of vancomycin.



Fig 4
Section of untreated globe 2 weeks after injection of methicillin resistant *S aureus* Note inflammatory exudate in vitreous and anterior chamber and signs of phthisis bulbi (hematoxylin and eosin ×9)

segment The control eyes showed a rapidly progressing panophthalmitis At the end of 2 weeks there was a diffuse opacity throughout the eyes and they were filled with pus Perilimbal hyperemia was also seen The control eyes all progressed to phthisis bulbi (Fig 4)

Of the 10 eyes treated with vancomycin only one eye progressed to destruction of the retina and phthisis bulbi In the nine remaining eyes there was an iritis that lasted for 1 to 2 days then resolved After 2 days the red reflex was present and 2 weeks later the media was clear and fundus detail was present There were no significant differences between the eyes treated 8 hours after infection (Fig 5) and those treated 12 hours after infection

Discussion

This study reaffirms the value of intravitreal injection in endophthalmitis Pryor Apt & Leopold (1962) were not able to achieve therapeutic vitreous levels by intravenous subconjunctival or topical administration of vancomycin but

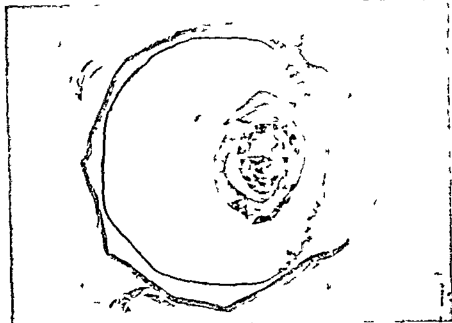


Fig 5
Whole section of eye treated intravitreally with 1 mg of vancomycin 8 hours after inoculation of methicillin resistant *S aureus* (hematoxylin and eosin ×9)

they did reach levels in the aqueous of 16 μg/ml after intravenous administration in rabbits with chemically inflamed eyes We achieved levels over twice as high (37 μg/ml) in the aqueous after intravitreal injection although it took 6 hours to reach those peak levels in the aqueous as opposed to 1 hour after intravenous injection in the study by Pryor Apt & Leopold

Even if vancomycin were as effective when administered systemically as when intravitreally administered the obvious disadvantages of systemic administration are possible nephrotoxicity and ototoxicity One valid argument against the administration of vancomycin intraocularly is the possibility of retinal toxicity In our study we showed that 1 mg of vancomycin appears to be nontoxic to the retina as well as to the rest of the eye In addition this dosage achieves greater than the needed concentration to eradicate most cases of staphylococcal endophthalmitis

With rapidly emerging new strains of drug resistant staphylococcus vancomycin may be the drug of choice for suspected cases of staphylococcal endophthalmitis

Material and Methods

The material and methods employed in this study were identical to those described in a previous investigation (Johnson 1974a) In brief 30 adult Dutch rabbits were anaesthetised with urethane A tracheostomy and a retrograde cannulation of the abdominal aorta via a femoral artery were performed Each anterior chamber was cannulated one of the cannulae being connected to a manometer and pump by which means it was possible to raise and maintain the intraocular pressure at any given level Ischaemia was produced in one eye of each animal by raising the intraocular pressure above systolic arterial blood pressure for either 15 30 60 90 or 120 min The pressure in the other eye remained normal throughout the experiment and this eye acted as a control In one group of animals two animals were sacrificed immediately after each period of ischaemia The remaining animals were either killed after 60 or 240 min after the return of normal intraocular pressure Two animals were killed at each recovery period from each individual period of ischaemia The eyes of the animals killed immediately following the period of ischaemia were enucleated bissected in the equatorial plane and the posterior halves immersed in fixative at room temperature In the remaining animals the eyes were removed following intra arterial perfusion with fixative The jugular vein was cut in these animals as an exit channel for the spent fixative and expelled blood The fixative in all cases was 3 % glutaraldehyde buffered with Sorensen s phosphate buffer Tissue was taken from the region of the visual streak periphery and horizontal nerve fibre layer and processed conventionally for electron microscopy 400–600A° thick sections were stained with uranyl acetate and lead citrate and examined with a Siemens 1A Elmiskop

Results

The endothelium of the choroidal vessels appeared to be relatively resistant to ischaemic damage induced by high intraocular pressure Immediately following the varying periods of ischaemia an occasional myelin body was observed (Fig 2a) These were 0 1–0 4 μ in diameter and composed of a few tightly packed membranes and material of high electron density The mitochondria

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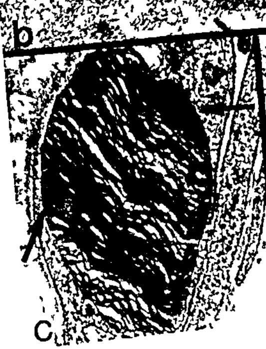
Fig 1

Electronmicrograph of the choroidal endothelium showing in lusion bodies (Ph) containing membranous debris (× 30 000)


Ph


mb
a


b


c


d

were frequently distended with a decrease in the electron density of the matrix and a shortening of the cristae There were also frequent lysosomal like bodies These were membrane bound and contained a granular material of high electron density (Fig 2a) In addition there appeared to be a decrease in the number of pinocytic vesicles in the ischaemic endothelium although no quantitative analysis was carried out In general these features became more prominent with increasing durations of ischaemia During the post ischaemic recovery phase the endothelium rapidly regained a normal appearance although there was occasionally cytoplasmic clefting (Fig 2b) and the mitochondrial matrix generally had a higher electron density than normal

In addition to the myelin bodies other inclusion bodies were evident in the endothelial cytoplasm (Figs 1 2b and 2c) These were membrane bound bodies 0 3–1 5 μ in diameter containing organised membranous material Apart from this material the phagosome contained discrete areas of granular material of high electron density (Figs 2b and 2c) The endothelial phagosomes appeared similar to the phagosomes encountered in the retinal pigment epithelium They were particularly similar in both size and organisation to the phagosomes during the initial stages of the pigment epithelium s disposal and degradation of the terminal portions of the visual cells outer segments

The choroidal endothelium was the only location in the choroid in which these phagosomes containing lamellar material were found They were absent from Bruch s membrane the vessel lumen and choroidal histocytes

In the 30 eyes examined following periods of ischaemia and post ischaemic recovery endothelial phagosomes were found in six preparations Of these six one was from the time immediately following the ischaemic episode and the remainder from various times during the post ischaemic recovery phase (Table 1)

Fig 2

(a) Electron micrograph of a typical mylein body (mb) in the choroidal endothelium Numerous lysosome like bodies (L) are also present (× 30 000)

(b) Electron micrograph of endothelial phagosomes containing membranous debris and possessing regions of reticular matter with high electron density (arrow) (× 30 000)

(c and d) Electron micrographs showing similarities in size shape and contents between an endothelial phagosome (arrow indicates reticular mass) (Fig c × 90 000) and a phagosome found in the retinal pigment epithelium (arrow indicates reticular mass) (Fig d × 90 000)

Table I
Table showing the distribution of the six preparations in which endothelial phagosomes were found

		Minutes recovery		
		0	60	240
	15	0	1	0
Minutes	30	1	1	0
Ischaemia	60	0	1	1
	90	0	0	1
	120	0	0	0

Discussion

Phagosomes have been shown to occur in the choroidal endothelium of the rabbit following periods of ischaemia Their presence and origin is open to much speculation

A similarity does exist between these endothelial phagosomes and those of the retinal pigment epithelium and the contents of the endothelial phagosomes may be of visual cell origin If this is so the material in the phagosomes must *reach the choroidal endothelium* either as a disrupted outer segment membrane material or as partly digested phagosomes from the pigment epithelium *In* either case one must postulate a *trans pigment epithelial route to the choroidal* vessels for even after lengthy periods of ischaemia the apical surface of the pigment epithelial cells remained intact and the pigment epithelium formed an intact layer over Bruch s membrane throughout the experiments The visual cell debris in whatever form may be liberated from the pigment epithelium via ruptures in it s basal cell wall which occur during recovery from periods of ischaemia (Johnson 1974b) and this may represent one possible ro te by which partly digested phagosomes may pass into the choroidal circulation No such material was observed in either Bruch s membrane or in the lumen of the blood vessels However the small chance of observing this latter feature must have been further reduced by the use of intra arterial perfusion during the process of fixation Partly digested phagosomes may also be able to pass out of intact pigment epithelial cells as Hogan (1972) described their presence in Bruch s membrane in the human eye

There appeared to be a clear distinction between the typical myelin body and the endothelial phagosome The contents of the latter contained many more membranous structures which were generally organised in a more *orderly*

fashion The presence of these phagosomes may indicate a latent phagocytic ability of the choroidal endothelium exposed by ischaemic insult

The vascular endothelium outside the reticulo endothelial system has been shown to be capable of phagocytosis although this does not occur readily Cotran (1965) has demonstrated in rats and mice that following repeated injections of colloidal carbon phagocytosis was evident in the endothelium of small myo cardial vessels in the endocardium in the pulmonary capillary endothelium in the aorta and in glomerular and peritubular capillary endothelium In the eye the non vascular endothelium lining the trabecular beams underlying Schlemm s canal is also capable of considerable phagocytic activity (Grierson & Lee 1973)

Under normal circumstances the choroidal endothelium does not appear to be phagocytically active Ischaemia induced by high intraocular pressure may be a sufficient stimulus to expose a latent phagocytic ability of the choroidal endo thelium This route for the removal of outer segment debris must account for only a small amount of the debris compared with the removal of similar material by macrophages (Johnson 1974a) and the retinal pigment epithelium (Johnson 1975)

Acknowledgements

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SMOKING AND INTRAOCULAR PRESSURE

BY

SAMI L BAHNA* and TOR BJERKEDAL

The intraocular pressure – as measured by Schiotz tonometer – was studied in 378 smokers 151 ex smokers and 495 non smokers It was found that the three groups have practically the same distribution of intraocular pressure and that the latter has no relationship to the smoking habit.

Key words smoking – intraocular pressure

Nicotine in small doses is known to have a sympathomimetic action and evokes the discharge of catecholamines from the adrenal medulla (Volle & Koelle 1971) In man sympathomimetic effects of nicotine on the cardiovascular system can be demonstrated by injecting 2 mg of nicotine intravenously (Volle & Koelle 1971) a dose which is equivalent to that contained in the smoke of an average cigarette In relation to the eye prolonged sympathetic nervous system stimulation results in a fall in the intraocular pressure (Jaffe 1948 Greaves & Perkins 1952) Many studies demonstrated that topical application of adrenaline or other catecholamines lowers the intraocular pressure by inhibiting the formation of aqueous humour and/or facilitating its outflow (Weekers et al 1955 Aasved 1964 Bhattacherjee 1971 Lorenzetti 1971 Noah et al 1971) Accordingly it

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may be expected that the intraocular pressure is lower in smokers than in non smokers The extensive literature on smoking and health is as yet – to the best of our knowledge – devoid of reports on this subject Hence the purpose of the present study to investigate the existence of any relationship between smoking and intraocular pressure

Material and Methods

The material of this study was obtained through a multiphasic Health Testing Programme *which since September 1968 has been offered to the employees of* the University of Bergen

Included in the programme is a questionnaire which is delivered to the employee a few days prior to the clinical investigation so that it can be completed in ample time Besides information on history of diseases and symptoms related to various systems of the body the questionnaire inquires about some habits and activities The section on smoking includes questions on form of tobacco smoking amount of tobacco consumed inhalation of smoke and date of onset of regular smoking

One of the many clinical measurements performed on each individual is tonometry This was carried out by a well trained nurse using a standardized Schiotz tonometer with a plunger load of 5 5 g The anaesthetic drops used were oxibuprocaine chloride 0 2 % All readings were taken to the nearest half scale unit – the right eye first then the left eye – by the same observer and same instrument and only during the daytime between 9 a m and 1 p m Individuals with intraocular pressure higher than 4 5/5 5 (i e 18 9 mmHg) were referred to the ophthalmologist for confirmation and further investigation

Data processing was complete for employees who were investigated for the first time during the period from September 1968 to October 1971 During this period intraocular pressure of 1 862 eyes (941 right and 921 left) was measured in 1 024 individuals between 16 and 70 years of age Total percentage of the eyes examined was 90 9 % the reading was not taken in some eyes because of either non cooperation of the person or a pathological eye lesion

In this study the individuals were classified according to the smoking status into three groups smokers *ex smokers and non smokers A smoker was defined* as the person who at the time of inquiry was regularly smoking one cigarette or more daily *An ex smoker* as the person who was not smoking at the time of inquiry and for at least 1 month earlier but used to smoke in the past at least one cigarette daily for a year *A non smoker* as the person who either never

Table I

Mean age sex distribution and eyes examined in groups of smokers ex smokers and non smokers

Smoking status	No of persons	Age Mean ± SD	Sex				Eyes examined	
			Males		Females			
			No	%	No	%	No	%
Smokers	378	36 5 ± 12 4	238	63 5	140	37 0	681	90 1
Ex smokers	151	40 8 ± 11 3	107	70 9	44	29 1	276	91 4
Non smokers	495	33 5 ± 10 5	276	55 8	219	44 2	905	91 4
Total	1024	35 7 ± 11 6	621	60 6	403	39 4	1862	90 9

smoked or casually smoked According to these definitions of the 1 024 investigated persons there were 378 smokers 151 ex smokers and 495 non smokers The mean age sex distribution and percentage of eyes examined in *each of these three groups* are presented in Table I

In data processing for individuals smoking hand rolled cigarettes the number of cigarettes per day was calculated by dividing the amount in grams of tobacco consumption per week by 7 the weight of an average cigarette is 1 g The intraocular pressure readings were converted from Schiotz/5 5 scale units to mmHg using the Calibration Scale of 1955 The findings were statistically evaluated at a level of significance of 0 05

Results and Discussion

From Table II it may be seen that *smokers ex smokers* and non smokers had similar ranges of intraocular pressure and practically the same average Also the most frequent scale reading was the same in the three groups – 5 0/5 5 (17 3 mmHg) Only a few eyes were found to have tensions higher than 4 0/5.5 (20 6 mmHg) which shows no relationship between ocular hypertension and smoking

Also the degree of skewness – as measured by the moment coefficient of

Table II

Range mean ± standard deviation and moment coefficient of skewness of intraocular pressure (mmHg) and eyes with hypertension in smokers ex smokers and non smokers

Smoking status	Range of intraocular pressure	Total eyes Mean ± SD	Eyes with IOP > 20 6 mmHg No	%	Moment coeff of skewness	Right eye Mean ± SD	Left eye Mean ± SD	The eye with higher press Mean ± SD
Smokers	12 9 to 24 4	16 2 ± 1 53	2	0 29	+ 0 19	16 1 ± 1 [illegible]	16 2 ± 1 55	16 7 ± 1 46
Ex smokers	10 9 to 24 4	16 2 ± 1 69	3	1 09	+ 0 73	16 2 ± 1 84	16 2 ± 1 08	16 7 ± 1 [illegible]6
Non smokers	11 2 to 22 0	16 1 ± 1 54	2	0 22	+ 0 38	16 1 ± 1 58	16 1 ± 1 30	16 6 ± 1 53

skewness – did not vary much between the three groups The distribution of intraocular pressure in each of the three groups was slightly positively skewed (i e towards higher tensions) a known characteristic of the distribution of intra ocular pressure (Graham & Hollows 1964 Davanger & Holter 1965 Aasved 1971)

The differences in age and sex distributions of the three groups have no effect in this study The intraocular pressure in the non smokers did not significantly vary with age or sex – a finding which has also been previously reported (Goedblood et al 1961 Davson 1963 Aasved 1971)

From the Table it can also be seen that in neither right nor left eyes did the *mean intraocular pressure vary with the smoking status* The same was noted when the eye with the higher tension was taken to represent the individual

In the smokers group the intraocular pressure showed no significant variation with the smoking habit – cigarettes pipe cigar or mixed (Table III) Neither was there a significant difference between any of these subgroups and the non smokers group

In the group of cigarette smokers the intraocular pressure was neither significantly correlated with the number of cigarettes consumed per day at the time of inquiry ($r = -0\ 06$) nor with the duration of smoking ($r = +0\ 03$)

Cigarette smokers using non filter tipped cigarettes showed a mean intra ocular pressure of 16 0 ± 1 62 mmHg and those who inhale the smoke showed a mean of 16 2 ± 1 52 mmHg both figures are very close to that in non smokers (16 1 ± 1 54 mmHg)

Table III
Mean ± standard deviation of intraocular pressure in the smokers group according to the smoking habit

Smoking habit	No of eyes	Mean ± SD (mmHg)
Cigarette	477	16 2 ± 1 55
Pipe	89	15 9 ± 1 79
Cigar	16	16 4 ± 1 16
Mixed	84	16 3 ± 1 15
Unknown	15	16 4 ± 0 98
Total	681	16 2 ± 1 53

Of the cigarette smokers 18 had reported to be smoking for 20 years or more inhaling the smoke and consuming at the time of inquiry 20 or more non filter tipped cigarettes a day In this particular group the mean intraocular pressure was 16 5 ± 1 82 mmHg which is not significantly different from that in non smokers

It appears from this study that intraocular pressure has no relationship with habitual tobacco smoking in any form or degree It may be that the sympathetic nervous system stimulation and the increase in blood adrenaline produced by nicotine intake through chronic smoking are not of sufficient degree to influence the formation or outflow of aqueous humour This view might be supported by the finding of Tucci & Sode (1972) that there was no significant difference between habitual cigarette smokers and non smokers with respect to excretion of catecholamines

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PERMEABILITY OF RABBIT CORNEAL EPITHELIUM TO HORSERADISH PEROXIDASE AFTER THE INFLUENCE OF BENZALKONIUM CHLORIDE

BY

ASBJØRN M. TØNJUM

A tight barrier against permeation of horseradish peroxidase into the corneal epithelium exists at the corneal surface adjacent to the tear film. The present light and transmission electron microscopic study reveals that the cationic surfactant, benzalkonium chloride, which is commonly added to eye drops as a preservative, breaks down this barrier. Lysis of the cell membranes was demonstrated, resulting in a leakage of the tracer into and underneath the superficial cells. The grade of cellular destruction caused by benzalkonium chloride was dependent upon the concentration and exposure time of the drug.

Key words: permeability – cornea – epithelium – horseradish peroxidase – cationic surfactant – benzalkonium – rabbit

In a previous paper Tønjum (1974) demonstrated that horseradish peroxidase having a molecular weight of about 40 000 did not enter the normal rabbit epithelium from the anterior side. A tight barrier was present between the most superficial cells adjacent to the tear film. A lesion involving only the superficial cell layer of the epithelium might therefore tend to increase the permeability of water solutes and even macromolecules.

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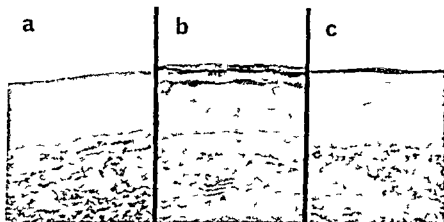


Fig 1

Light micrographs of rabbit corneal epithelium embedded in Epon and stained with toluidine blue a) Epithelium exposed to horseradish peroxidase *in vitro* for 10 min. b) Epithelium exposed to 0 02 % benzalkonium chloride for 5 min and to horseradish peroxidase for 10 min *in vitro* Disintegration of superficial cells and peroxidase reaction products inside the cells at several layers c) Epithelium exposed to BA 0 01 % for 2 min and horseradish peroxidase for 10 min Disintegration of the most superficial cell layer with peroxidase reaction products inside these cells × 470

The cationic surfactant benzalkonium chloride (BA) which is commonly used as a preservative in eye drops has been shown to increase the action of drugs applied topically (O Brien & Swan 1942 Swan 1944) and the permeability of fluorescein across the cornea *in vitro* (Green & Tønjum 1971) It has also been demonstrated that BA enhances the penetration of prednisolone phosphate into the eye *in vitro* (Green & Downs 1974) Another cationic surfactant cetyl pyridinium chloride has been shown to increase the permeability of isolated frog skin (Webb 1965)

The purpose of the present paper was to investigate whether benzalkonium chloride would influence the permeability of the corneal epithelium to the protein molecule horseradish peroxidase

Materials and Methods

Healthy adult albino rabbits weighing 3–4 5 kg were used Their eyes were found to be normal prior to experimentation.

The *in vitro* studies were done in a similar manner as described by Tønjum (1974) The excised corneas were clamped between two lucite chambers and BA

at a concentration of 0 02 % in Krebs Ringer solution was placed in the epithelial side chamber for 5 min and 0 01 % BA for 1 or 2 min After these exposure periods the BA was removed and this chamber was washed with Krebs Ringer solution The epithelial side chamber was then filled with Krebs Ringer solution to which was added horseradish peroxidase Sigma type II (PO) at a concentration of 2 5 mg/ml and left for 10 min The same procedures were done in parallel experiments the only difference being the exclusion of BA The endothelial side chamber was filled with Krebs Ringer solution throughout the experiment

The *in vivo* experiments were done by applying BA at a concentration of 0 02 % in Krebs Ringer solution topically into a rabbit eye one drop 3 or 10 times with 30 sec intervals Thereafter one drop of PO in Krebs Ringer solution at a concentration of 2 5 mg/ml was applied every half min for 10 min

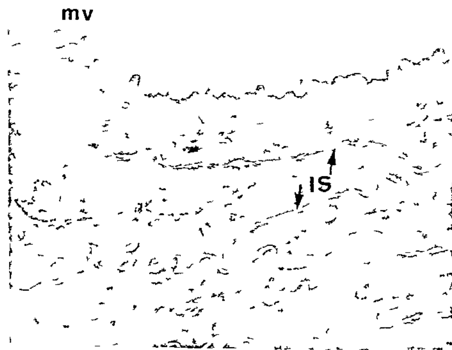


Fig 2

Normal rabbit epithelium exposed to horseradish peroxidase for 10 min *in vitro* No peroxidase reaction products underneath the surface Stained with alcaline lead MV microvilli IS intercellular spaces Numerous dark glycogen granules × 24 000



Fig 5

Rabbit *corneal* epithelium exposed to benzalkonium chloride 0 02% *in vitro* for 5 min and horseradish peroxidase for 10 min Disintegration of several cell layers with peroxidase reaction products inside the superficial and the wing cells Stained with alcaline lead × 18 000

The corneas were then removed from the chambers or excised from the animals which were killed with an overdose of sodium pentobarbital. The corneas were quickly washed in Krebs Ringer solution and prefixed in 2½ % glutaraldehyde. The tissues were then incubated with diaminobenzidine (DAB) and hydrogen peroxide, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon 812.

Semithin sections of about 1 μm in thickness for light microscopy were cut with an LKB Ultratome and examined either stained or unstained with toluidine blue. The ultrathin sections were either left uncontrasted or were contrasted with alcaline lead and/or uranyl acetate, and electron micrographs were taken with a Siemens Elmiskop 1 A.

Observations

The in vitro experiments

a) *Exposure of 0.02 % BA to the corneal surface for 5 min.* There was a gross disintegration of the cells in the superficial epithelium with numerous defects of the cell membrane at the surface (Figs. 1 and 4). The cytoplasma had leaked out of the cells, whereas PO was demonstrated inside. The intercellular spaces were widened and the cell membranes were stretched corresponding to the desmosomes, probably due to influx of fluid. Accumulations of circular structures were found particularly at the site near the nucleus (Fig. 3). In the wing cell layer the architecture of the cells was fairly well preserved with only slight intercellular oedema. However, large amounts of peroxidase reaction products (PORP) were present within the cytoplasma and the nuclei. Between the well preserved deep wing cells and the basal cells there were traces of PORP, but no gross intercellular oedema.

b) *Exposure of 0.01 % BA to the corneal surface for 2 min.* The ultramicroscopic changes were in principle equal to those of the previous experiment, but were limited to the most superficial cell layer. This was, however, completely disorganized with defects of the anterior cell surface, presence of PORP inside these cells, and lysis of the intracellular organelles including the nuclei (Fig. 5). In the deeper layers PORP were demonstrated in the intercellular spaces (Fig. 6).

c) *Exposure of 0.01 % BA to the corneal surface for 1 min.* The structural changes found in these experiments were moderate. Even some cells of the corneas not treated with BA appeared to be dead and contained PORP. In the present set of experiments some more cells appeared to be dead, and others had relatively few microvilli (Fig. 7).

in eye drops after prolonged exposure to the eye produces a disintegration of the cellular architecture and function The degree of the changes was dependent upon the concentration and exposure time of the BA

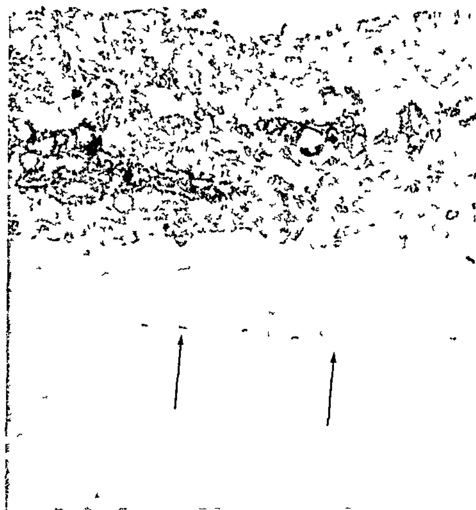


Fig 6

Rabbit corneal epithelium exposed to benzalkonium chloride 0 01 % for 2 min and horseradish peroxidase for 10 min *in vitro* Uncontrasted Peroxidase reaction products within the superficial cells and between the deeper cells *(arrows)* × 18 000

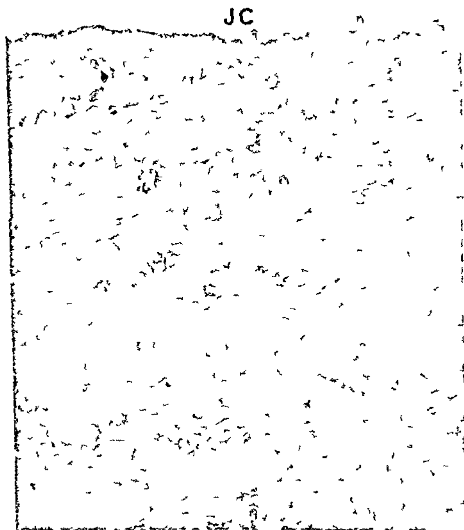


Fig 7

Rabbit corneal epithelium exposed to benzalkonium chloride 0 01 % for 1 min and horseradish peroxidase for 10 min *in vitro* No gross lysis of cell membranes or organelles JC tight intercellular junctional complex The left cell has a straight surface with few microvilli The right one appears undamaged Alcaline lead × 20 000



Fig 6

Rabbit corneal epithelium after topical application of benzalkonium chl[illegible]de 0 0[illegible] 10 times with 30 sec intervals and horseradish *peroxidase* [illegible]0 times *in* [illegible] Cross lysis of the superficial layers Alcaline lead 18 000

It is difficult to compare the effect of BA in the *in vivo* and *in vitro* experiments but it appeared that the cornea *in vivo* was more resistent to the destruction caused by BA than *in vitro* even taking into account the dilution of the drug which takes place *in vivo* A possible explanation for this might be the protective effect of the tear film *in vivo*

The *in vitro* experiments showed that exposure to 0 01 % BA for 2 min damaged cells of the entire surface Exposure for 1 min left some cells relatively unharmed whereas others were affected Exposure to BA 0 01 % for 1 min *in vitro* therefore seems to approach the lethal toxicity of BA to corneal epithelial cells That all cells are not affected to the same degree may be due to different vulnerabilities to BA in different stages of the life cycle of corneal epithelial cells which have a high turnover rate

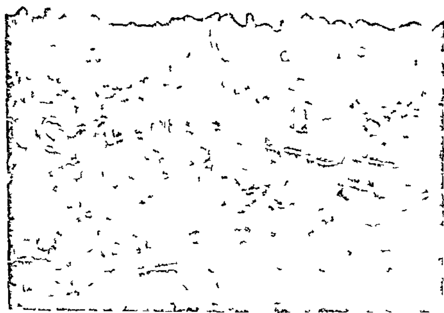


Fig 9

Rabbit corneal epithelium after topical application of benzalkonium chloride 0 02 % 3 times and horseradish peroxidase 90 times *in vivo* No gross damage of the ultrastructure The microvilli seems less prominent than in epithelium untreated with benzalkonium Alcaline lead × 94 000

Even when only the superficial cell layer is damaged the barrier function of the epithelium is broken down and PORP could be found in the intercellular spaces of the deep cell layers (Fig 6) The PORP staining might have been more intensive with a longer exposure time for PO Another problem is visualizing PO in the deeper layers when the tissues are incubated *en bloc* because of the poor penetration of diaminobenzidine to these tissues

In the superficial cells isolated or aggregated circular structures or vesicles were present They may reflect the general tendency of membranes to form globules when being disintegrated or they may be swollen intracellular organelles

The mechanism by which BA breaks down the permeability barrier appears to be the lytic effect upon the cellular membrane and the intracellular membranes as well allowing fluid and solutes to enter the cells Eventually the disintegration reaches the stage when the cell contents leak out leaving a shell consisting of a defect cell membrane

It is reasonable to assume that long term clinical use of BA enchances the turnover rate of the corneal epithelial cells It might also be assumed that BA penetrates into the deeper corneal tissues affecting other cells such as the keratocytes and endothelial cells On the other hand in some clinical conditions it might be an advantage at least for a limited period of time to enhance the penetration of a drug into the eye particularly the water soluble drugs It is also important to be aware of BA activity when comparing the effects of different drugs upon the eye

Acknowledgement

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THE EFFECT OF BENZALKONIUM CHLORIDE ON THE ELECTROPOTENTIAL OF THE RABBIT CORNEA

BY

KEITH GREEN and ASBJORN M TØNJUM

The effect of benzalkonium chloride on the electropotential of the cornea has been examined The anterior surface of the *in vivo* or *in vitro* cornea was exposed to various concentrations of the surfactant from 0 005 % to 0 02 % for either 1 or 2 min The initial effect is a hyperpolarization lasting up to 30 sec followed by a rapid fall in potential difference with a subsequent recovery The degree of potential difference decrease and the recovery rate was dependent upon both the concentration of the detergent and the exposure time There is excellent correlation between the previous anatomical and physiological studies on tracer penetration across the *in vivo* and *in vitro* cornea and our present work The data indicate that benzalkonium chloride acts by breaking down the physiological and anatomical diffusion barrier to solute and solvent which is located in the outer layer of the epithelium

Key words benzalkonium chloride – cationic surfactant – rabbit – cornea electropotential

Benzalkonium chloride (BA) a widely used preservative in ophthalmic preparations has been shown to enhance the permeability of the rabbit cornea *in vitro* to both fluorescein (Green & Tonjum 1971) and a large molecule horseradish peroxidase (Tonjum 1975)

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The normal corneal epithelium is impermeant to horseradish peroxidase (PO) when the tracer is applied to the anterior surface When applied posteriorly it moved between the epithelial cells to the tight junctional complexes adjacent to the tear film between the most superficial cells (Tønjum 1974) thus demonstrating that the barrier is located at the surface This is in agreement with Green (1969) who showed that the anterior part of the epithelium is more resistant to water movement than the posterior layer and with electrophysiological studies of Ehlers (1973) and Klyce (1973)

The tracer technique with PO demonstrated that BA *in vitro* produced defects of the cell membranes allowing PO to pass into and through the superficial cells

O Brien & Swan (1942) found that benzalkonium chloride increased the *in vivo* effect of carbachol upon the intraocular pressure Lund Karlsen & Fonnum (1975) showed increased intraocular penetration of cholinesterase reactivators after topical BA application *in vivo* Green & Downs (1974 a b) demonstrated that BA enhanced the ocular penetration of prednisolone phosphate and pilocarpine

In light of these previous findings an investigation was made of the effect of BA on the electropotential of the cornea and the rate of possible recovery of the potential on both the *in vivo* and *in vitro* rabbit cornea

Materials and Methods

Adult healthy albino rabbits 2–4 kg and of each sex were used

In vitro experiments

The rabbits were killed with an overdose of sodium pentobarbital administered via a marginal ear vein An eye was proptosed and the cornea together with a scleral rim removed and placed in Krebs bicarbonate Ringer with glucose added at 5 mg/ml (hereafter referred to as Ringer) The iris and lens were then gently removed and the isolated corneas mounted in chambers as described previously by Green (1966) These chambers allow a hydrostatic pressure head (15 mmHg) to be placed on the posterior surface of the cornea The chamber was immersed in a beaker containing sufficient Ringer to cover the exposed corneal surface All experiments were performed at room temperature

The potential difference (PD) across the cornea was recorded with one of two systems A Heath EU 20B recorder with a voltage clamp attachment, or a Keithley Model 160 Digital Multimeter Both systems are capable of measuring PD to an accuracy of ± 0 1 mV with 10 mV full scale deflection With the Keithley

multimeter agar/saturated KCl (made in PE160 tubing) connected the solutions bathing each surface of the cornea to beakers also containing saturated KCl and the connection to the voltmeter was made via Beckman calomel electrodes Using the Heath recorder the agar bridges connected the bathing solutions directly to calomel electrodes and these connected to the recording system

Experiments were performed using normal Ringer initially on both sides of the cornea At 15 min after mounting the tissue in the chamber all tissues exhibited a stable control PD An identical Ringer solution containing either 0 005 0 01 or 0 02 % benzalkonium chloride (BA) was then placed in another beaker and the chamber placed in this solution for either 1 or 2 min At the end of this exposure to BA the chamber was returned to a beaker containing Ringer alone The PD across the cornea was measured continuously during all phases At least four corneas were used at each concentration and exposure time

In vivo experiments

The rabbits were anesthetized with intravenous urethane (25 % w/v in 0 9 % NaCl) After a suitable depth of anesthesia had been induced a corneal cup which had a height of 2 cm and a diameter of about 1 cm was placed on the cornea The rabbit was placed on its side for this procedure and only one eye per animal was utilized to avoid problems associated with exposure or abrasion of the contralateral cornea The cup had a rim at the bottom to fit at the corneal limbus and produce a leak proof fit with the eye Ringer was placed in this cup and the PD across the cornea measured between an electrode placed in this solution and an electrode inserted into a marginal ear vein The agar/KCl electrodes used in these experiments were made of PE 50 tubing to facilitate entry of the electrode into the vein

After a steady PD was recorded the Ringer in the cup was replaced by an identical solution containing BA at 0 005 or 0 1 % for 1 min and this was further replaced by normal Ringer The PD was measured almost continuously during all these phases using a Heath EU 20B recorder with a voltage clamp attachment After the first 10 min following reapplication of normal Ringer to the cornea the PD was measured at 10 min intervals with the corneal cup removed from the eye and the lids held together with a clamp when PD was not measured

Results

Some results obtained from exposure of isolated cornea to benzalkonium chloride (BA) are presented in Table I where certain trends are evident BA usually

Table I

Values of hyperpolarization percentage reduction and recovery of the corneal potential difference after exposure of the anterior surface of the *in vitro* cornea to various concentrations of benzalkonium chloride for 1 or 2 min. The time of minimum PD is given as the time at which the PD reached a minimum to the time at which higher values were consistently found. The recovery of original PD is the value found 2 hours after exposure to the surfactant

Concentration and time		Hyper polarization % of original PD	Maximal reduction % of original PD	Recovery after 2 hours % of original PD	Time of minimum PD (min)
0 005 %	1 min	10	13	91	15
	2 min		64	86	5 to 10
0 01 %	1 min	45	50	95	5 to 20
	2 min		81	50	10 to 60
0 02 %	1 min	51	80	63	5 to 30
	2 min		91	18	5 to 60

caused an initial concentration dependent hyperpolarization of the cornea This response was short lasting with the potential difference (PD) peaking between 20 and 30 sec after exposure to BA The PD then fell rapidly in most cases in a concentration dependent manner to a minimum The decrease in PD was also greater with longer exposure times (Table I)

The PD recovered at varied rates towards normal pre treatment levels when returned to Ringer solution The recovery rate was also exposure time and concentration dependent (Table I) It is immediately evident that a lower concentration for 2 min is almost equivalent to a higher concentration for 1 min

In vitro experiments

0 005 % BA One minute exposure of rabbit cornea to BA resulted in a small reduction of PD from 3 97 ± 0 30 (SEM) mV with the maximum fall at 15 min to 3 48 ± 0 18 mV ($P < 01$) (Fig 1 a) The average hyperpolarization for 1 and 2 min was 0 4 mV The average maximum reduction in PD was 13 % PD recovery was 91 % of the control value at 2 hours

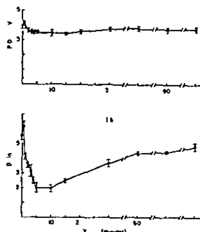


Fig 1

Effect of 0 005 % benzalkonium chloride on the electropotential of the isolated rabbit cornea PD potential difference (mV) time in minutes O——O 1 min exposure to BA ●——● 2 min exposure to BA. Values are the mean ± SEM

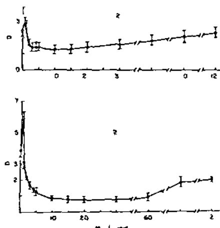


Fig 2

Effect of 0 01 % benzalkonium chloride on the electropotential of the isolated rabbit cornea. For explanation of symbols see legend to Fig 1

Two minute exposure to BA caused a significantly greater reduction of PD from 5.55 ± 0.35 mV with the maximum fall from 5 through 10 min to 2.0 ± 0.2 mV ($P < 0.01$) (Fig 1 b) The average maximum reduction in PD was 64 % PD recovery was 86 % of the control value at 2 hours (96 % at 3 hours)

0.01 % BA One minute exposure caused a fall in PD from 2.45 ± 0.35 mV with a maximum fall from 5 through 20 min to 1.23 ± 0.26 mV ($P < 0.01$) (Fig 2 a) The hyperpolarization for 1 and 2 min was 1.1 mV The average maximum reduction in PD was 50 % PD recovery was 95 % of the control value at 2 hours

Two minute exposure caused a fall in PD from 3.9 ± 0.4 mV with a maximum fall from 10 through 60 min to 0.75 ± 0.15 mV ($P < 0.01$) (Fig 2 b) The average maximum reduction in PD was 81 % PD recovery was 50 % of the control value at 2 hours (77 % at 3 hours)

0.02 % BA One minute exposure caused a reduction of PD from 2.35 ± 0.24 mV with a maximum fall from 5 through 30 min to 0.45 ± 0.16 mV ($P < 0.01$) (Fig 3 a) The hyperpolarization for 1 and 2 min was 1.2 mV The average maximum reduction in PD was 80 % PD recovery was 63 % of the control value at 2 hours

Two minute exposure caused a reduction of PD from 2.45 ± 0.18 mV with a maximum fall from 5 through 60 min to 0.25 ± 0.17 mV ($P < 0.01$) (Fig 3 b) The average maximum reduction in PD was 91 % PD recovery was 18 % of the control value at 2 hours

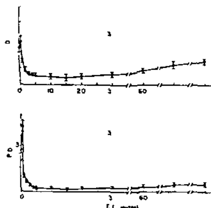


Fig 3

Effect of 0.02 % benzalkonium chloride on the electropotential of the isolated rabbit cornea For explanation of symbols see legend to Fig 1

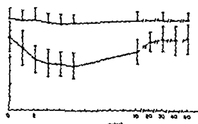


Fig 4

Effect of benzalkonium chloride on the electropotential of the *in vivo* rabbit cornea PD potential difference (mV) time in minutes O——O 0 005 % for 1 min ●——● 0 01 % for 1 min

In vivo experiments

One minute exposure of the cornea to 0 005 % BA caused a small depression in PD from 6 9 ± 0 8 to 6 5 ± 0 7 mV ($2 < P < 01$) The fall in PD is 5 ± 3 % ($2 < P < 1$) and is not significantly different from the control value

One minute exposure of the *in vivo* rabbit cornea to 0 01 % BA caused a fall in PD from 5 5 ± 1 1 to 3 4 ± 1 0 mV ($P < 01$) The fall in PD is 35 ± 8 % ($P < 001$) and is significantly different from the control value The time of maximum decrease is at 5 min (Fig 4) with recovery of the PD to control values at about 20 min

Discussion

The PD of the isolated rabbit cornea shows a characteristic behaviour after exposure to BA At high doses the PD increases within the first 30 sec and this hyperpolarization is followed by a marked fall The magnitude of the fall is both exposure time and concentration dependent Low (0 005 %) concentrations only elicited small hyperpolarizations a minor fall in PD and recovery of PD is complete within 30 to 60 min As the concentration increases the initial hyperpolarization and the fall in PD are increased and the subsequent recovery is longer Longer exposure times also extend the length of the maximum fall and the extent of recovery of the PD (Table I)

The cornea of the living rabbit also responds to BA when added to the anterior corneal surface (Fig 4) The fall in PD after 0 01 % BA for 1 min is however less than that elicited by the same concentration *in vitro* (36 % compared

to 50 %) and the effect is of shorter duration Thus there is a difference between the *in vivo* and *in vitro* effects of the cationic surfactant

Anatomical evidence (Tønjum 1974) indicates that the normal corneal epithelium is impermeant to horseradish peroxidase (PO) After treatment of the *in vitro* cornea with BA PO penetrates into and between the surface cells of the epithelium After treatment of the *in vivo* cornea with BA and PO simultaneously there is an effect of the surfactant but compared to the *in vitro* effects the tracer penetration is not as marked (Tønjum 1975) There is therefore good agreement between the anatomical and electrophysiological data since the *in vitro* effects are more marked than the *in vivo* effects The anatomical evidence indicates that BA acts by inducing a lysis of the membrane of the outer cell layers of the epithelium thus removing the barrier function of these cells (Tønjum 1975) The electrophysiological and anatomical data indicate that the PD is reduced by a breakdown of this barrier which creates a shunt pathway for the movement of solutes and solvent

These findings are substantiated by other work on both *in vitro* and *in vivo* effects of BA Previously we have described the effects of BA on fluorescein permeability of the *in vitro* cornea where 4 min exposure of the cornea to 0 01 % BA caused a 12 fold increase in fluorescein transfer (Green & Tønjum 1971) *In vivo* studies using radioactive prednisolone sodium phosphate and pilocarpine (Green & Downs 1974 a b) reveal that BA 0 01 % causes only a 50 % increase in drug penetration 15 min after the administration of one drop (50 µl) to the eye Lund Karlsen & Fonnum (1975) examined the entry of cholinesterase reactivators into the eye and found that BA caused a significant increase between 31 and 126 % in drug penetration

The *in vivo* values indicate less enhancement in permeability than found with the same concentration of BA *in vitro* Our current electrophysiological data *in vivo* reveal decreases in PD which are less than those found in the *in vitro* cornea The recovery of the PD is much more rapid *in vivo* and again is presumably related to a quicker recovery of barrier function

The recovery of the PD is of importance since it reveals that the cornea is capable of undergoing regeneration of the barrier function of the membrane even *in vitro* Recovery of the barrier function may be in a manner similar to that observed in the normal cornea when dead pre desquamating cells offer no resistance to the entry of PO across the cell boundaries Although the cell is leaky PO does not pass beyond this cell and the anatomical barrier is established posterior to the dead cells (Tønjum 1974) Re establishment of the barrier function may be made posterior to the lysed superficial cells in the recovery phase of the present experiments

The present experiments resemble those made on toad bladder using another

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EFFECTS OF BENZALKONIUM CHLORIDE UPON THE CORNEAL EPITHELIUM STUDIED WITH SCANNING ELECTRON MICROSCOPY

BY

ASBJØRN M TØNJUM

The corneal surface of rabbits and vervet monkeys was studied by means of scanning electron microscopy after the influence of the cationic surfactant benzalkonium chloride The concentration of the drug was 0 02 % or 0 01 % and the exposure time was 2 or 4 min The corneas treated with the drug had characteristic changes with small holes or more extensive lesions of the superficial cell membranes and loss of microvilli or microplicae The damage was considered to be due to the lytic effect of benzalkonium chloride upon the plasma membranes

Key words benzalkonium chloride – surfactant – detergent – cornea – epithelium – scanning electron microscopy – permeability

The epithelium is a barrier which prevents water solutes and large molecules entering the cornea The most important location of this barrier has been shown to be the anterior surface of the epithelium the superficial plasma membranes and the intercellular junctional complexes between the most superficial cells (Tonjum 1974)

Benzalkonium chloride when applied to the anterior corneal surface breaks down this barrier presumably by producing defects of the plasma membranes

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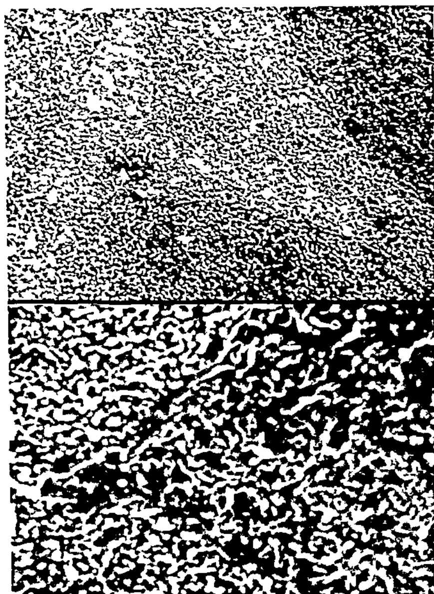


Fig 1

Surface of normal rabbit cornea showing excrescences Intercellular borders are present
A ×3 000 B ×10 000

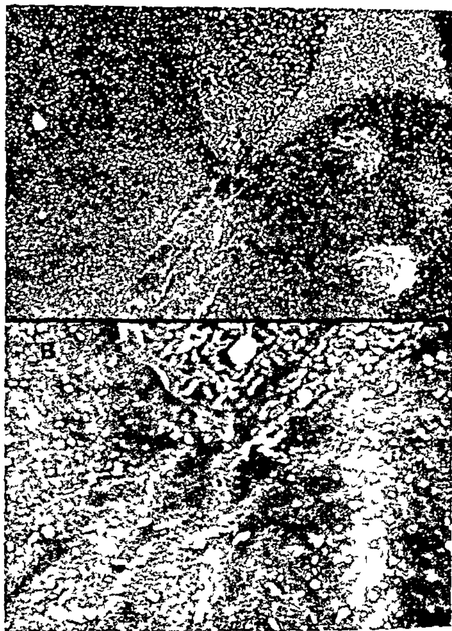


Fig 3

Surface of rabbit cornea exposed to 0 01 % benzalkonium chloride for 2 min A Different degrees of damage in the different cells Loss of surface excrescences One cell is apparently undamaged × 3 000 B The same specimen as above with small defects of the plasma membrane × 10 000

b *The corneal surface after exposure to BA 0 02 % for 4 min* (Figs 2 A 2 B 5) At low magnifications the surface was smooth At higher magnifications the surface of all cells were seen to be devoid of excrescences and to have defects of varying degrees In some cells the entire anterior plasma membrane was disintegrated whereas others had smaller defects At high magnifications the smallest holes were circular and had a diameter of about 20 nm (Fig 5)

c *The corneal surface after exposure to BA 0 01 % for 2 min* (Figs 3 A 3 B 4 A 4 B) In principle the damage was similar to that of the previous experi



Fig 4

Surface of monkey cornea A Normal epithelium B Cornea exposed to 0 01 % benzalkonium chloride for 2 min. Smooth surface with loss of surface excrescences × 3 000

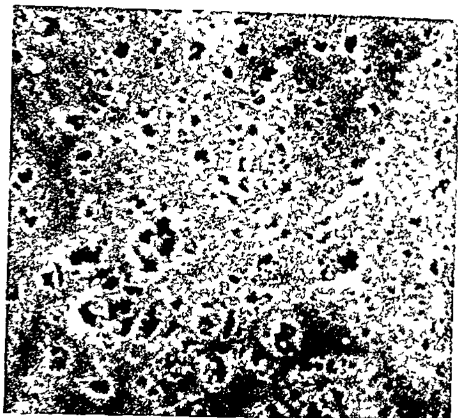


Fig 5

Surface of rabbit corneal epithelium exposed to 0 02 % benzalkonium chloride for 4 min Penetrating defects of the plasma membrane Some holes are isolated the smallest ones having a diameter of about 20 nm others are confluencing × 30 000

ment, although less pronounced One particular feature was the difference in damage of the different cells from severe disintegration to almost non detectable damage (Figs 3 A 3 B) The presence of small holes however was a consistent finding in all cells In some places these appeared to have confluenced to larger defects In the monkey eye the damage seemed more severe than in the rabbit

Comments

The effects of BA upon the corneal epithelium have been studied by means of scanning electron microscopy The main findings were a) loss of surface excrescences b) penetrating defects of the superficial cell membrane The degree

of damage was dependent upon the concentration of BA and the duration of the exposure of this solution to the corneal surface

There was a difference in the response of the cells to the influence of BA this was particularly seen in the series of experiments when the corneas were exposed to 0 01 % BA for 2 min In the series when the corneas were treated with BA 0 02 % for 4 min all cells were severely damaged The turnover rate of epithelial cells is high and the differences of vulnerability may reflect the different stages in the life cycle of the cells Many surface cells of the corneas not treated with BA had defects of the plasma membrane too However these cells did not have the conspicuous loss of surface excrescences

The surface coating by the tear film most likely influenced the appearance at least of those corneas not treated with BA This cationic surfactant exerts an effect both upon the lipid and the mucinous components of the tear film Possibly the tear film is partly or totally lost by the BA treatment However it was beyond the scope of the present work to rule out this effect

The penetrating holes of the plasma membranes are most likely a primary effect of BA They were fairly easily differentiated from the defects of the surface cells of corneas not treated with BA Sites of the plasma membranes might be particularly sensitive to the effect of BA The intracellular disintegration (Tonjum 1975) may well be secondary to lysis of the plasma membranes and to effects upon the membranes surrounding the intracellular organelles as well Release of lysosomal enzymes may however upgrade the process of tissue destruction Furthermore Bangham & Horne (1964) demonstrated that surfactants produced holes in phospholipid globules Evidence of an early effect upon the membranes is also given by Saladino Hawkins & Trump (1971) who showed an increase of ion leakage in the toad bladder after the influence of another cationic surfactant cetyl pyridinium chloride Hodes Palmer & Warren (1960) demonstrated entrance of the dye nigrosin into Ehrlich ascites cells after the influence of surface active agents The possibility is therefore unlikely that the defects of the plasma membranes are secondary to intracellular damage

The loss of surface excrescences was a characteristic finding after BA treatment Most likely BA affects the appearance of the surface partly by acting upon the tear film *Furthermore the defects of the plasma membrane may result* in a reorganization of this structure at an early stage during the experimentation even though critical point drying gives an optimal preservation of the architecture of the cells The loss of surface excrescences could also be caused by the lytic effect of BA at the base of the excrescences In this case the holes of the plasma membranes could represent the previous sites of the excrescences

The observations by SEM that BA produces penetrating defects of the plasma membranes of the corneal epithelial cells and loss of surface excrescences support

the studies of the increased corneal permeability given by BA (O Brien & Swan 1942 Green & Tonjum 1971 Green & Downs 1974 Tonjum 1975) The holes of the cell membranes or the more extensive lesions offer an explanation for the mechanism of this permeability increase

Acknowledgements

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INTRAMUSCULAR RABBIT ANESTHESIA KETAMINE HYDROCHLORIDE AND MEBUMALNATRIUM (NFN)

A Safe and Easy Combination

BY

ERIK KROGH

For the attainment of deep anesthesia in rabbits a combination of ketamine hydrochloride (Ketalar®) and mebumalnatrium (NFN) (Nembutal®) administered intramuscularly was evaluated The anesthetic effect was satisfactory and no deaths or side effects were recorded

Key words ketamine hydrochloride – mebumalnatrium (NFN) – rabbit anesthesia – intramuscular anesthesia

A deep and prolonged intravenous anesthesia of rabbits demands considerable experience in the judgment of the dose of maintenance and in the care of the frequently arising cardiovascular and/or respiratory complications

In search of an easier method ketamine hydrochloride (2 (0 chlorophenyl) 2 (methylamino) cyclohexanone hydrochloride) (Ketalar®) seemed promising It is a short acting anesthetic for intramuscular application and as a rather special characteristic it stimulates respiration and circulation in pharmacological doses

Its draw backs are a deficient relaxation of the muscle tone and a small effect on visceral pain In some persons it provokes a characteristic EEG pattern which has led to speculation on the possible epileptogenic effect of Ketalar but

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a recent study by Corssen et al (1974) rejects such a hypothesis Consequently a deep anesthesia cannot be obtained with Ketalar alone but possibly in combination with a conventional anesthetic in moderate dosage As no report on such a combination anesthesia was found in the literature a trial is presented

Method and Results

The following scheme was used Ketalar 50 mg/kg and mebumalnatrium (NFN) (Nembutal®) 30 mg/kg in separate syringes This provides a deep anesthesia for 60–80 min with persistence of the corneal reflex only When excitation begins the anesthesia can be renewed with 30–50 % of the initial doses The recovery is slow but undramatic

In 20 adult albino rabbits no deaths or signs of hypoxia developed during 60–150 min of deep anesthesia In some cases with prolonged surgery and high body temperature electrolytic fluids were administered subcutaneously Airway hypersecretion did not occur consequently no atropine or oxygen were supplied

Conclusion

A combination of Ketalar and Nembutal is very suitable for a safe and easy intramuscular anesthesia of rabbits It permits eye surgery in a fully relaxed animal for at least 60–80 min without having to pay attention to cardiovascular and respiratory functions The influence on the intraocular pressure has *not* been studied but experience with Ketalar alone in man seems to indicate a (significantly ?) higher mean intraocular pressure compared to halothane anesthesia but attempts to confirm this difference in the rabbit were unsuccesful (Adams 1973)

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CONCENTRATIONS OF SOME METABOLITES IN THE AQUEOUS HUMOUR OF HUMAN SENILE CATARACTOUS EYES

BY

A BRUUN LAURSEN

Glucose pyruvate l lactate and citrate concentrations of the aqueous humour were examined in 51 patients aged 54–87 years with senile cataracts and in one patient with a clear lens Furthermore the ratios of glucose in aqueous/glucose in plasma and citrate in aqueous/citrate in plasma were investigated

Among the parameters recorded age dependence could only be demonstrated for glucose concentrations in the aqueous humour as this parameter decreases with increasing age However a chance significance because of multiple significance tests cannot be left out of account in this material The parameters in question are unfit for an evaluation of possible changes in the energy metabolism of the lens in relation to cataract development because no significant differences were found between concentrations and ratios in different clinical types of cataract Also considerable ranges of variation were recorded

Key words glucose – pyruvate – l lactate – citrate – aqueous humour – senile cataracts

In 1974 Bruun Laursen & Lorentzen published a paper on glucose pyruvate and citrate concentrations in the aqueous humour of fasting and non fasting patients with senile cataracts The ratios of glucose in aqueous/glucose in plasma

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and citrate in aqueous/citrate in serum were also examined to some extent Indications were found that the citrate concentrations of the aqueous as well as the ratios of citrate in aqueous/citrate in serum increased when cataract lenses grew totally opaque However reservations were made for chance significances because of the multiple significance tests performed the levels of significance being only 0 05 $> P >$ 0 02

The purpose of the present paper is to re investigate the aforesaid parameters in the aqueous humour of fasting patients suffering from senile cataracts and to compare the concentrations and ratios of the different immature cataract groups with the corresponding values of patients having totally opaque lenses extracted Thus we hope either to confirm or invalidate the results of the previous paper

In addition l lactate and the ratio l lactate/pyruvate will be introduced as parameters in this publication

Material

Aqueous humour was analysed from 51 fasting patients aged 54–87 years with senile cataracts Patients with additional eye disease diabetes mellitus and patients undergoing ocular or universal treatment with corticosteroids or drugs of the digitalis group during the period of cataract development were excluded from this material

The aqueous was aspirated during cataract operation just before the anterior chamber was opened Needles no 26 G 3/8 were used for puncturing The *cataract operations were performed in facial akinesia and retrobulbar* anaes thesia with carbocain (mepivacaini chloridum NFN) without norepinephrine

Ear capillary blood fluoridated and heparinized was used for plasma glucose determinations Cubital vein blood was used for serum citrate determinations The samples were analysed immediately

Methods

The aqueous humour was immediately heated to boiling point by means of a *spirit flame in order to destroy glycolytic enzymes* (Bruun Laursen & Lorentzen 1975)

For glucose determinations a Beckman glucose analyser was used (Kadish et al 1968)

For pyruvate citrate and l lactate concentrations Biochemica Boehringer were used – with certain changes (Bruun Laursen & Lorentzen 1975) However the pH of the lactate buffer was raised from 7 8 to 8 5 recoveries thereby rising to nearly 100 % Thus using buffer pH 7 8 the median recovery of 3 00 mmol l lactate/l in watery solution (19 determinations) was 2 4 mmol/l whereas using buffer pH 8.5 the median recovery of 3 00 mmol l lactate/l in watery solution (19 determinations) was 2 9 mmol/l – range of variation = 0 4 mmol/l The highest value recovered was 3 0 mmol/l For serum citrate determinations 0 5 mol/l of triethanolamine buffer pH 7 6 was used

For classification of senile cataracts the following terminology was used

Group 1 posterior subcapsular
Group 2 posterior subcapsular + deep cortical
Group 3 posterior subcapsular + nuclear
Group 4 posterior subcapsular + anterior subcapsular
Group 5 posterior subcapsular + more than one of the other types
Group 6 totally opaque lenses
Group 7 nuclear
Group 8 deep cortical
Group 9 clear lens (melanoma eye)

Results

The results appear in Table I By means of the Wilcoxon test for two samples groups with four or more sets of results were statistically compared with group 6 (totally opaque lenses) this group thus serving as reference group Neither the glucose concentrations of the aqueous (median = 3 1 mmol/l) the ratios of glucose in aqueous/glucose in plasma (median = 0 64) the pyruvate concentrations of the aqueous (median = 258 μmol/l) the l lactate concentrations of the aqueous (median = 5 0 mmol/l) the ratios of l lactate/pyruvate of the aqueous (median = 20 1) the citrate concentrations of the aqueous (median = 116 μmol/l) nor the ratios of citrate in aqueous/citrate in serum (median = 0 96) of the various groups of immature cataracts differed significantly from the values of eyes with totally opaque lenses

Values of one patient with a localized choroidal melanoma and a clear lens resembled values of cataractous patients

For these reasons the cataract material was treated as one group in Table I

The parameters in question were tested for age dependence by means of the Spearman coefficient of rank correlation A significant correlation was found

Table I (cont)

	Group	Age years	Gluc aq mmol/l	Gluc pl mmol/l	Ratio gluc aq / Gluc pl	Pyruvate aq µmol/l	l Lactate aq mmol/l	Ratio lactate / pyruvate	Citrate aq µmol/l	Citrate serum µmol/l	Ratio citrate aq / citrate serum
	6	74	3 0	4 7	0 64	251	5 3	21 1	112	97	1 16
	6	79	2 7	5 4	0 50	250	5 5	22 0	118	193	0 61
	7	78	3 9	5 1	0 76	254	3 8	15 0	106	78	1 36
	8	79	3 1	4 4	0 70	280	4 7	16 8	106	167	0 63
	8	85	2 4	4 8	0 50	211	5 5	26 1	116	92	1 26
	8	93	2 6	4 3	0 60	219	4 6	21 0	81	139	0 58
	8	69	3 2	5 6	0 57	223	5 1	22 9	84	81	1 04
	8	82	3 1	4 9	0 63	238	5 2	21 9	105	151	0 70
	8	56	3 4	5 0	0 68	267	4 9	18 4	110	39	2 80
Medians		73	3 1		0 64	253	5 0	20 1	116		0 96
10 % percentiles		59	2 4		0 50	205	3 8	15 8	97		0 64
90 % percentiles		83	4 2		0 81	285	5 6	23 5	149		1 36
Ranges of variation		33	2 6		0 51	151	2 8	15 1	98		2 28
	9	57	2 6	4 7	0 55	217	4 9	22 6	114	69	1 65

solely for glucose concentrations of the aqueous humour which were found to decrease with increasing age ($0.05 > P > 0.02$). Thus the median for 10 patients aged 65 years or less was 3.2 mmol glucose/l and for 10 patients aged 80 years or more the median was 2.9 mmol/l.

Discussion

The median glucose concentration in the aqueous of this material (3.1 mmol/l) is consistent with the values reported by Bruun Laursen & Lorentzen (1974) where the means for 17 fasting cataractous patients were 3.1 mmol/l and for 10 non fasting patients 3.9 mmol glucose/l.

The same is true of the ratios of glucose in aqueous/glucose in plasma, the medians of the latter values being 0.64 in this work and 0.6 both in fasting and non fasting patients in the aforementioned paper (Bruun Laursen & Lorentzen 1974). Pohjola (1966) found glucose values in the aqueous ranging between 2.1 and 4.8 mmol/l in 20 patients suffering from senile cataracts. The ratios of glucose in aqueous/glucose in plasma ranged from 0.405 to 0.734 in Pohjola's material. Also Pohjola (1966) found no difference between the glucose concentrations in the aqueous humour of normal and cataractous eyes.

The pyruvate concentrations of the present material (median = 238 µmol/l) are higher than those reported by Bruun Laursen & Lorentzen (1974) where the median of 17 fasting patients was 152 µmol/l and that of 22 non fasting patients was 210 µmol/l. The higher pyruvate figures in the present material are in part due to the fact that reduction of the concentration of reduced nicotinamide adenine dinucleotide to 150 µmol/l in the cuvettes effected an increase in recoveries of both pyruvate and citrate in watery solutions from about 80 % to about 90 %.

De Berardinis et al. (1965) reported a mean lactate concentration in the aqueous humour of 17 human eyes with clear lenses of 4.28 mmol/kg ± SD = 1.30. In cataractous eyes the mean lactate concentration in the aqueous of 20 eyes was 4.69 mmol/kg ± SD = 0.90. The corresponding plasma mean values were 1.78 mmol/kg ± SD = 0.80 and 2.07 mmol/kg ± SD = 0.50, respectively. The median l lactate concentration in this material is 5.0 mmol/l.

The citrate concentrations of the aqueous in the present paper (median = 116 µmol/l) are consistent with those previously reported by Bruun Laursen & Lorentzen (1974) where the medians were 112 µmol/l in the aqueous of 17 fasting and 93 µmol/l in the aqueous of 22 non fasting patients with senile cataracts. Also the ratios of citrate in aqueous/citrate in serum of this material

(median = 0 96) are consistent with the figures of the previous paper (median = 0 93) in 21 patients (Bruun Laursen & Lorentzen 1974)

It appears reasonable to compare citrate concentrations of cubital vein serum with citrate concentrations in the aqueous humour as Lennér (1934) found by means of the Thunberg technique that the serum concentrations were independent of glycolytic and muscular activities Moreover he found about the same citrate value in arterial and cubital vein serum

Also it appears reasonable to compare glucose concentrations of ear capillary plasma with glucose concentrations of the aqueous humour However it is difficult to imagine any relationship between pyruvate and l lactate in the aqueous humour on one hand and the corresponding concentrations in cubital vein plasma on the other hand because of the probable influence of muscular glycolysis even with the slightest of movements on these metabolites Besides the aforementioned findings of Berardinis et al (1965) that aqueous humour lactate concentrations are 2–3 times higher than lactate concentrations of plasma indicate that aqueous humour lactate concentrations are only slightly influenced by plasma lactate levels

As for glucose and citrate nothing is gained by determining the ratios of concentration in aqueous/concentration in plasma The ranges of variation were not smaller than those of the aqueous concentrations alone and these parameters did not yield any new information Nor did the application of the ratio l lactate/pyruvate in the aqueous as a parameter yield any new information

The interindividual ranges of variation are large for the parameters in question as inaccuracies of the methods cannot account for them solely (Bruun Laursen & Lorentzen 1975) cf the glucose concentrations of the aqueous humour seem to decrease with increasing age However the statistical significance for this correlation is not high ($0\ 05 > P > 0\ 02$) and the possibility of an error of the first kind because of the multiple significance tests performed cannot, of course, be excluded It should be mentioned however that Pohjola (1966) demonstrated decreasing glucose concentrations in the aqueous humour of humans ranging between 20 and 80 years of age

Bruun Laursen & Lorentzen (1974) found indications of increasing ratios of citrate in aqueous/citrate in serum and increasing citrate concentrations in the *aqueous humour when lenses grew* totally opaque However they made reservations for errors of the first kind because of multiple significance tests the levels of significance being only $P < 0\ 05$ The present material points to chance significances in our first series The transparency of the lens does not affect glucose pyruvate l lactate or citrate concentrations in the aqueous humour no significant differences having been demonstrated between these parameters in eyes with different types of immature senile cataracts on one

hand and eyes with totally opaque lenses on the other hand Thus no information is obtainable about possible changes in lens metabolism in relation to cataract development by means of the parameters used in this material Direct investigations into the contents of lens energy metabolites *seem to be necessary if a possible* correlation between senile cataracts and defects in lens energy metabolism is to be explored

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OBJECTIVE RECOGNITION OF ABNORMAL ISOPTERS

BY

LARS FRISÉN and MARIANNE FRISÉN

We have examined the hypothesis that normal and only normal isopters of the central visual field are elliptical except for random deviations Statistical methods capable of recognizing small deviations from elliptical shape have been developed These methods have been devised to meet the special demands of clinical testing of visual fields and require only simple calculations The qualities of these methods were examined by theoretical analysis by large scale simulations and by experimental comparison with subjective evaluations The new methods proved very useful in these examinations

A clinical study using a servo equipped Goldman perimeter demonstrated that normal central isopters are elliptical enough for the proposed test characteristic to be of practical value in clinical work. The method allows an objective recognition of abnormal isopters with a high degree of sensitivity

Key words visual field – perimetry – isopters – statistical analysis – objective techniques – elliptical shape analysis

Detection of localized disturbances in peripheral vision is the primary goal of clinical perimetry In kinetic perimetry this ordinarily involves the mapping of loci where moving light stimuli are at threshold the fitting by eye of isopter curves connecting loci of equal sensitivity and the examination of the isopters

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for deviations from normal shape However meaningful definitions of normal isopter shapes are not available The sensitivity of the perimetric procedure or its detection potential is dependent on ill defined subjective factors and is likely to be variable (Frisen 1970 a)

Because perimetry involves subjective responses and a large number of more or less well controlled variables perimetrists have despaired of the possibility of applying a formal analysis to the results of the examination. The sources of variation do not by themselves prevent an application of statistical logic, how ever What is required for a successful procedure is a concise formulation of features differentiating normal results from abnormal

An attractive hypothesis is that normal isopters and only normal isopters belong to one and the same family of mathematical functions for instance the family of ellipses A feature differentiating normal isopters from abnormal ones would then be the failure of abnormal isopters to satisfy criteria for ellipticity A statistical tool capable of discriminating elliptical from non elliptical forms in the presence of stochastic (random) variation would thus be appropriate Such an analytical procedure applied to data obtained from currently recommended examination routines offers the possibility of enhancing the sensitivity of the perimetric method (Frisen 1970 a)

The suggestion that normal isopters may be elliptical except for stochastic deviation is not unsupported The general symmetry of the eyeball suggests that normal isopters should have a symmetrical configuration It seems particularly relevant that iso density plots of retinal ganglion cell distributions are said to be elliptical in outline (Van Buren 1963) Although less extensively documented iso density curves for rods and cones may share this feature (Østerberg 1935) When ellipses are subjected to the cartographic projection and deformation involved in perimetry the resulting curves show a remarkable similarity to average normal isopters (Frisen 1970 b) Furthermore using a perimetric set up partially eliminating cartographic distortion it has been possible to demonstrate that normal isopters of intermediary size do not significantly deviate from an ellipse characteristic viz diametrical symmetry (Frisén 1970 a)

This paper presents further evidence for an elliptical shape of normal isopters in the central field of vision Statistical procedures capable of recognizing small departures from elliptical shape have been developed Full details of the statistical theory are given elsewhere (Frisen 1974 a b) only a brief description of clinically important facts is given here The statistical tests were applied to a series of normal individuals as well as a series of computer simulated quadrant depressions of various types The latter procedure aimed at determining the sensitivity of the analytical procedure The results were compared with those of subjective evaluation

Only central isopters were studied in the first clinical trial because the problems of cartographic deformation are negligible in the central visual field The actual field examinations were made with a standard Haag Streit Goldmann perimeter equipped with a servo attachment ensuring freedom from examiners bias

Materials and Methods

Subjects The subjects were all untrained volunteers mostly medical students and medical personnel None had evidence of ocular or visual pathway disturbances Ages ranged between 18 and 60 years ametropias between –7 and +4 diopters No subject had an astigmatic error exceeding 1 diopter

The majority of the subjects had only one eye examined Left and right eyes were about equally represented

Perimetry A standard 940 Haag Streit Goldmann perimeter with four neutral filters was used The target projection system was provided with an adjustable iris diaphragm allowing a continuous graduation of target intensities (Frisen 1972) as the number of built in filters frequently proved too small to give isopters of the desired size The target size used throughout this study was No I 0 25 mm The blind spot was delineated using the same target size at maximum intensity (1000 asb) The background luminance was 31 5 asb

The target movements were controlled by means of a synchronous motor attachment (Frisen 1972) This device ensures linear pathways and constant rates of indicator movement The target speed used here was 1 5° per sec On perceiving the target the subject pressed a hand held microswitch instantly bringing the movement to a stop The position of the target at this moment was taken as the observation

The statistical design (see below) requires that all target trajectories intersect on the long isopter axis In a pilot study (described below) it was found that this axis regularly cut the vertical meridian 1.3 degrees of angle below the point of fixation The meridians of the perimetric chart can thus be used to define target trajectories only if the lon isopter axis is made to go through the pole of the chart This transposition can be made either by using a fixation mark 1 3° above the customary fixation mark or by displacing the recording chart 1 6 mm downwards The latter alternative was used here

Following 5–10 min of adaptation and a suitable number of practice presentations of the target the absolute blind spot was charted in a centrifugal fashion Eight observations were made corresponding to horizontal vertical and mid quadrantic chords all through the same origin In the ensuing isopter charting the appropriately dimmed target was presented along selected chart meridians using the same rate of movement. The various meridians were always examined in a centripetal diametrical fashion in a predetermined order designed to preclude training and fatigue bias Fixation was frequently checked

The subjects were provided with an appropriate correction for ametropia and/or presbyopia

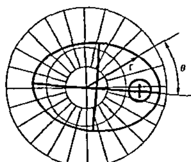


Fig 1
Elliptical model of a normal isopter The long isopter axis runs through the pole and the center of the blind spot

Statistical model

Considering the sources of variation (e g reaction time fixation errors number of light quanta absorbed) it is judged reasonable to adopt for the observations of an isopter the model

$$r_i = f(\Theta \;\; A) + e_i \qquad (1)$$

where r_i is the length of radius No i Θ the meridian angle (Fig 1) A is a vector of unknown parameters and e the random component In this model the random components for different observations are independently distributed all with the expected value zero It is also judged reasonable to assume that the random components are normally and identically distributed or at least approximately so (Frisén 1974 b) f is a function for which the set of polar coordinates (r Θ) represents a simple closed continuous curve when $r = f(\Theta)$ In particular $r = f \; (\Theta)$ will denote a circle and $r = f_e\,(\Theta)$ will denote an ellipse

A test of the hypothesis f = f is needed both to decide whether the shape of normal isopters is elliptical or not and to discriminate between elliptical (normal) shape and the shapes of defective isopters Standard statistical methods do not apply to this problem but appropriate methods can be based on some special characteristics of ellipses These methods will be described in the following They all used information on the location of the center of the blind spot

Observations and statistical strategies

Central isopters (temporal radius 8–25°) were obtained from 77 normal individuals during the development of the standardized technique These isopters

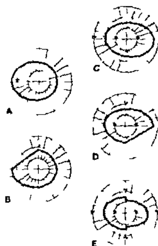


Fig 2
Models of normal (A C) and abnormal isopters (B D E) of different sizes and proportions These models with superposed stochastic deviation were used in the simulations

were not used in the statistical shape analysis but served to illuminate the response variation along the different meridians the position of the center of gravity the axis ratio and the axis position Similar studies were made on the blind spot.

On inspection these isopters always appeared compatible with the ellipse hypothesis except for random deviations A superposed ellipse with individually appropriate axis lengths with the long axis passing through the center of the blind spot and a point 1 3° below the point of fixation always seemed to give a good fit to the observations The axis ratio of the ellipse and the position of its center showed marked inter individual variation. Elevating the upper eyelid above its physiological position did not appreciably affect the isopter characteristics

Statistical tests capable of recognizing relevant shape parameters amidst stochastic (random) variation were then developed on the basis of geometrical properties of true ellipses A true ellipse has several potentially useful shape characteristics An exactly defined relationship between parts of intersecting chords (i) and reflection symmetry with respect to the long ellipse axis (ii) were studied in particular and will be further detailed in the following

Normal and abnormal isopters were simulated in an IBM 360 computer Two *locations of isopters were used central and intermediary in the* visual field.

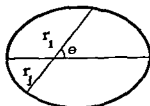


Fig 3
Variables used in the chord method

For normal isopters observations were generated on the basis of ellipses with the orientations and proportions estimated from the pilot study for isopters of these sizes (Fig 2 A C) Stochastic response variation was superposed along meridians according to the statistical model (1) The standard deviations were 1 51 and 1 72 degrees of arc respectively For each isopter size 2 000 isopters were generated The statistical tests for reflection symmetry and chord part relationship were applied to these two sets of normal data and to similar sets where one or two ellipse quadrants had been indented slightly before introducing the stochastic variation (Fig 2 B D E) This procedure aimed at illuminating accuracy and sensitivity of the tests information that would be impossible to obtain from clinical studies

The localized depressions were by design so shallow that about 25 % escaped detection in a pilot study using ordinary subjective evaluation

(i) The chord method It can be proved that the two parts r and r_j of a chord cutting one ellipse axis (Fig 3) are related by

$$\frac{1}{r_j} - \frac{1}{r} = C \cos \Theta = S \qquad (2)$$

where C is a constant for the ellipse (Frisén 1974 b) Thus S /cosΘ is the same for all chords through one and the same point on the axis of an ellipse The relation (2) has been studied when r of an exact ellipse is replaced by E(r) in stochastic deviation as described by model (1) Simulations under several conditions of special interest in the present application have not revealed any departures from the relation (2) of importance in relation to the variance Several statistical methods based on the relation (2) and capable of recognizing deviations from elliptical shape have been compared with respect to their ability to correctly classify one single set of observations (i e one single isopter) The statistical power i e the probability of detecting the given deviations from elliptical shape was determined by means of simulations

Table I

The power corresponding to a 5 % significance level in the analysis of variance of the ratio S The theoretical calculations were based on the assumption that all conditions for the analysis are fulfilled exactly

Isopter (cf Fig 2)	Power determined by			
	Theoretical calculations		Simulations	
	n = 2	n = 3	n = 2	n = 3
A (ellipse)	0.050	0.050	0.049	0.050
B	0.13	0.24	0.13	0.25
C (ellipse)	0.050	0.050	0.066	0.073
D	0.37	0.71	0.37	0.069
E	0.35	0.69	0.36	0.63

Table I shows the power corresponding to a 5 % significance level of the versatile analysis of variance of the ratio S Two or three observations (n) were simulated on each of 12 evenly spaced meridians Each estimate of the power was based on 2 000 simulated isopters

Randomly drawn samples of 25 simulated normal and 25 abnormal observa

Table II

Comparison between subjective judgement and analysis of variance of the ratio S on the 5 % level Twenty five isopters of each kind were evaluated each comprising two simulated observations for each of 12 values of Θ

Isopter (cf Fig 2)	Fraction judged as defective by					
	Judge no				Average subjective judgement	Analysis of variance
	1	2	3	4		
C (ellipse)	0.08	0.04	0.12	0.16	0.10	0.08
E	0.76	0.68	0.64	0.84	0.78	0.72

tions were also presented to two senior ophthalmologists who were asked to judge whether each simulated isopter was normal or abnormal in shape. Their performance was compared to the performance of the chord test. Similarly two medical laymen (statisticians) were asked to judge whether each chart was compatible with an ellipse except for stochastic deviations.

A comparison between the subjective evaluation and the analysis of variance is given in Table II. Two observations for each of 12 values of θ were simulated for each isopter. The results of the objective procedure are remarkably similar to those of careful unhurried subjective judges.

(ii) The reflection symmetry test. The ellipse exhibits reflection symmetry with respect to its axis. When the origin is located on an axis

$$E(r_i - r_j) = 0 \quad \text{for } \theta_i = -\theta_j$$

It is not very practical to choose θ so that $\theta_i = -\theta_j$ because this requires estimation of the location of the blind spot center during the actual examination of a patient. The conventional examination procedure involves determination of r from meridians spaced at angles of 15 or 30° apart using the horizontal meridians as reference. The long axis of an isopter is inclined with respect to the horizontal meridian and in general the meridians where observations are obtained are not symmetrically distributed with respect to this axis. Thus two consecutive observations ordered by cosθ will belong alternatingly to the upper and the lower half of the isopter. This suggests another characteristic, namely the number (U) of runs up and down of r ordered by cosθ. For $f = f_e$ the expected number

Table III

The power of the reflection symmetry test as reflected by the fraction of rejected isopters for various critical regions. The first three columns apply when it is known a priori in which isopter half a defect may occur, the latter columns apply when no such information is available.

Isopter (cf. Fig. 2)	$U \geq$			$\max(U_L, U_T) \geq$		
	9	10	11	9	10	11
A (ellipse)	0.230	0.067	0.012	0.447	0.135	0.022
B	0.80	0.56	0.25	0.85	0.60	0.26
C (ellipse)	0.134	0.036	0.005	0.177	0.107	0.018
D	0.93	0.82	0.49	0.95	0.83	0.42

Table IV

Comparison between subjective judgements and the test of reflection symmetry Twenty five isopters of each kind were evaluated each comprising 24 evenly spaced simulated observations

Isopter (cf Fig 2)	Fraction judged as defective by					
	Judge no				Average subjective judgement	$\max(U_N, U_T) \geq 10$
	1	2	3	4		
A (ellipse)	0 44	0 24	0 20	0 12	0 25	0 12
B	0 80	0 76	0 44	0 82	0 71	0 72

of runs up and down of r ordered by $\cos O$ is not larger than that for a sequence of random order However for deviations of the kind illustrated in Fig 2 B D the number is larger

The power of the reflection symmetry test was illustrated by the percentage of 2 000 isopters which was rejected with a critical region of runs $(U) \geq 9$ 10 and 11 respectively Twenty four observations were simulated for each isopter The results from each isopter half (U_N and U_T where N and T represent the nasal and temporal halves respectively) must be analysed simultaneously if it is not known a priori where to suspect a defect The larger of U_N and U_T [maximum (U_N U_T)] is used as the test characteristic (Frisén 1974 b) (Table III) A comparison between subjective evaluation and the reflection symmetry test is summarized in Table IV Twenty five isopters were randomly selected and each was evaluated by the four subjective judges Only one of the four judges (none of the ophthalmologists) made more accurate classifications than the reflection symmetry test The others erred particularly on the false positive side

Because of the rarity of visual field defects that are reflection symmetrical the uniquely simple reflection symmetry test seems ideally suited for routine clinical use The amount of calculation involved is literally minimal All that is required once the observations are obtained is determination of the center of the blind spot construction of the long isopter axis running through this point and the chart pole folding of the chart along this line transferring observations from one isopter half to the other counting the number of runs up and down (Figs 4 and 5) and comparison with a critical value (Table III) The appropriate

critical level depends on the situation It is never possible to discriminate small defects from normals without error The use of a high critical region (i e U ≧ 11) results in a very small risk of erratic classification of normals but also in a poor ability to detect defective isopters The use of a low critical region (i e U ≥ 9) has the opposite effect The properties of different critical values for another number of observations than that used here (24) cannot be determined without new simulations However the probabilities for a completely random order (Owen 1962) are upper bounds for the probabilities of erroneously rejecting normal isopters

The estimation of the center of the blind spot is a problem from the statistical point of view as a good estimate requires knowledge of the shape of the blind spot A series of observations on the shape of the normal blind spot were made in an earlier study (Frisen 1970 a) using eccentric fixation in the perimeter The point of fixation was translated so as to center the blind spot within the pole of the perimeter this procedure makes cartographic deformation of the blind spot negligible (Frisen 1970 b) Under these conditions the shape of the blind spot appears circular except for random deviations A test of a hypothesis of circular shape was therefore performed The test was based on the geometrical theorem of circle chords In the presence of stochastic deviation this means that

$$E(r_i r_j) = C \text{ for } \Theta_j = \Theta_i + 180 \tag{4}$$

where C is a constant for the circle The constancy in relation (9) was tested by a runs up and down criterion. No deviation from circular shape was detected although the

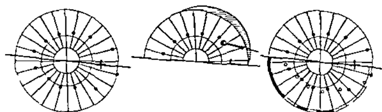


Fig 4

A method of symmetry testing The visual field chart (to the left) is folded along the line through the pole and the center of the blind spot The observations on the upper half are then transferred to the lower half by means of a pin (middle illustration) The markings on the lower half are now alternatingly original and transferred observations (illustration to the right) Comparing their distances from the chart pole in order from the left (counterclockwise) it can be seen that the first four markings form a down run (dark stripe in the margin of the chart) nos 4 and 5 an up run (light stripe) nos 5 and 6 a down run and so on There are four runs in the left half of the isopter and two in the other half in this example

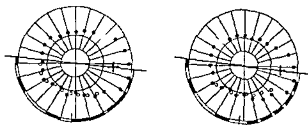


Fig 5

Constructed examples of normal and abnormal numbers of runs. The set of observations to the right are generated from an isopter with a shallow upper temporal depression. Maximum (U_N, U_T) is 6 and 11 respectively.

sample size was large enough to ascertain a considerable power (> 0.98) against an ellipse with an axis ratio of $\sqrt{2}$.

An unbiased and effective estimate of the position of the center of a circle was constructed for use in routine estimation of the center of the blind spot. A Cartesian system of coordinates with the origin in the point of intersection of the target trajectories is used. The coordinates (α, β) of the center is estimated by

$$\alpha = 2\overline{X} \qquad \beta = 2\overline{Y}$$

where $\overline{X}$ and $\overline{Y}$ are the averages of the X and Y coordinates of the observations. It can be shown that this estimate is useful even if the shape of the blind spot is elliptical rather than circular, as long as the axis ratio is $< \sqrt{2}$ (Frisén 1974 b).

Clinical test of the shape of normal central isopters. It was desired to test statistically whether normal central isopters and ellipses are similar enough to prevent a rejection of too large a proportion of normal individuals. It was considered that normal isopters deviate too much from elliptical shape if 10% or more can be expected to be rejected when a testing method is applied at the 5% level of significance. The power to detect the deviation from elliptical shape just defined was set at 0.90. The risk of concluding that normal isopters deviate too much from elliptical shape when in fact they are perfectly elliptical was set at 0.01.

A sequential probability test with these properties was constructed using approximations when necessary (Frisén 1974 b). The design has the limit for acceptance $\Sigma U = -8.51 + 7.91\,n$ and for rejection $\Sigma U = 19.24 + 7.91\,n$ where U is the test characteristic (number of runs) and n is the number of isopters tested.

The plan of the test and its results are given in Fig 6. The results demonstrate that normal central isopters are elliptical enough for the suggested test characteristic to be of practical value in clinical work.

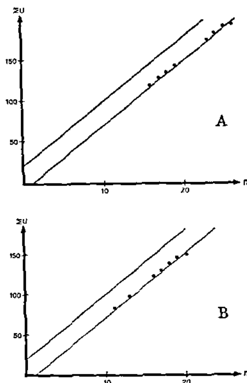


Fig 6

Plan and results of the sequential analysis of central isopters from normal individuals A and B apply to the nasal and temporal isopter halves respectively The upper line in each diagram defines the rejection limit the lower line the acceptance limit The points represent summed observed values

Discussion

The sensitivity of perimetry – its ability to discriminate between normal and abnormal visual fields – is intimately related to how well the characteristics of a normal visual field are known Previous attempts towards defining normal results were based on group examinations The resulting limits were too wide to be of clinical value Experience shows that while the actual linear dimensions of normal isopters for given stimuli vary considerably the shapes of these curves seem to be more constant. Perimetrists have therefore long preferred shape criteria rather than dimensional parameters for distinguishing normal results from abnormal obviously this would have been impossible if normal curves did

not share common characteristics of shape An *efficient use of such shape* characteristics means that interindividual size variation can be disregarded, each individual being evaluated only on the basis of whether or not within his range of variation he meets the examiner s criteria of normal shape

Previously this evaluation has been purely *subjective quantitative* perimetry being quantitative only on a technical instrumental basis With a subjective evaluation of results the sensitivity of the perimetric method is likely to be highly variable and less than maximal This is true for both static and kinetic perimetry It is unlikely that the *invention of new perimetric techniques* will improve on this situation However objective and truly quantitative procedures applied to the evaluation of results have this potential By defining for central isopters of kinetic perimetry a shape characteristic that can be examined by *procedures of statistical logic we have been able to demonstrate the possibility* of improving the performance of the perimetric method without actually changing the technique of examination or the format of records This shape characteristic also provides an improved standard of comparison for subjective evaluation *However this is a less effective procedure than statistical testing* It is known what the statistical test measures and how well it performs

The most easily utilized characteristic is the one based on reflection symmetry as it involves no more than a count of runs up and down after folding and *piercing the visual field chart and comparison with a tabulated critical* value for the desired level of significance In spite of its unique simplicity this test proved to be at least as sensitive as careful subjective evaluation on large samples of computer simulated quadrant depressions of various magnitudes yet *the rate of false positives was lower The single drawback with* this test is that it is unable to detect depressions that are symmetrical to the long isopter axis However exactly symmetrical defects of this type are in our experience very rare in clinical practice

The reflection symmetry test should be replaced by an analysis of variance of chord part relationships if axis symmetrical depressions are of special interest The analysis of variance requires replicated perimetric observations (say three observations on each meridian tested) This disadvantage is offset by the fact *that fewer meridians need to be explored in the detection of the types* of defects studied here With access to a programmable desk calculator the actual computation is very simple Although this test is capable of detecting axis symmetrical defects also for the same number of observations it is slightly less sensitive than *the reflection symmetry* test for asymmetrical depressions The latter test is thus probably the more useful one Both procedures are considerably simpler than the one developed by Fankhauser et al (1972) and Koch et al (1972) However the goals and conditions of the two approaches are quite different Firstly the

method developed by Fankhauser and associates requires static perimetry while the present procedure requires kinetic perimetry Secondly the primary aim of Fankhauser s procedure is to detect scotomas of relatively small area It is not suitable for shallow defects such as relative quadrant defects The analytical procedure developed here has the opposite aims and qualities The two thus supplement each other

It must be emphasized that the visual field examination must be unbiased for a meaningful application of statistical logic. Statistical tests are unable to differ entiate between abnormalities arising from poor examination techniques and those attributable to the subject The rules of perimetry must be strictly adhered to This is probably best accomplished by using a target movement control device e g a servo attachment (Frisen 1972) Statistical tests are also unable to provide a differential diagnosis This part of the evaluation procedure re mains subjective This also applies to the identification of asymmetrical normal variants including baring of the blind spot and temporal cuts associated with obliquely inserted optic discs In this context it is interesting to note that a retinal center for functional symmetry lies above the macula This has a counter part in the orientation of the optical axis of the eye (Brown 1972)

For reasons given in the introduction it seems likely that peripheral isopters also share the shape characteristics of central isopters The cartographic de formation involved in perimetric recording of results increases with increasing isopter size however and outside the central part of the field it may require correction when the analysis of variance test is applied Studies of the properties of non central isopters will also require attention to the effects of drooping eyelids obscuring facial contours etc The operation of complicating factors like these may at least partially explain the notion that liminal studies of the peripheral part of the visual field very rarely disclose diagnostic information that is not available in selective central field studies (Blum et al 1959)

As the number of meaningful responses that can be obtained from a subject in one sitting is quite limited it seems a wise screening strategy to concentrate on the central visual field and to use all aids available to find any abnormalities An examination procedure of the type used here combined with objective and sensitive statistical tests for the analysis of results is a logical approach to the problem of efficient truly quantitative visual field screening

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QUADRANT SPARING OF THE MACULA

BY

NIELS EHLERS

Macular sparing is an indisputable clinical finding in cases of hemianopia Several explanations have been given some of which are not mutually exclusive Eight cases encountered in a study of more than 100 hemianopias are presented in which the sparing has the form of a quadrant, either upper or lower The explanation in these cases seems to be the double vascular supply to the tip of the occipital pole It is stressed however that macular sparing may have a different explanation in other cases

Key words quadrant sparing – macular sparing – vascular supply – occipital pole

The sparing of the macula is a frequent finding in homonymous hemianopia It is a phenomenon which has been and continues to be much debated All authors seem to agree upon its existence as a clinical finding but as regards its explanation opinions are numerous Walsh & Hoyt (1969) state with complete assurance that there is as yet no satisfactory explanation for it Possible causes of the sparing are

1 Cortical area of the macula preserved due to partial destruction only of an occipital lobe

2 Macula represented in large occipital area some undamaged macular area being left (Porsaa 1944)

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3 Double vascular supply to the tip of the occipital pole (Traquair 1917)

4 Excentric fixation and instability of fixation (Verhoeff 1943)

5 Defect is a hemiamblyopia Only the more resistent macular area has retained function (Rønne 1911)

6 Bicortical representation of each macula by (i) two fibres from each cone one passing to each hemisphere (ii) dichotomy of fibres in chiasma or (iii) a retrochiasmal commisural tract (Heine 1900)

7 Bilateral foveal representation that means receptors for the two hemispheres intermingled in the retinal fovea (Morax 1919 Dubois Poulsen et al 1952 Gramberg Danielsen 1959)

8 Artefact due to unstable fixation

These possibilities are more or less well defined and some of them may to a *certain extent, overlap one another In addition they are by no means mutually* exclusive There seems to be no reason to search for a single correct explanation for the sparing phenomenon The explanation may vary from one case to *another*

In this report cases are presented which show a structured sparing of the fixation area in the form of a quadrant sparing These cases which were *encountered in a study of more than 100 hemianopias support the explanation* based on a double vascular supply to the occipital pole viz the posterior cerebral artery on the medial aspect of the lobe and ramifications from the middle cerebral artery from the lateral aspect

Case histories

Case 15 (020499) 73 year old man with arterial hypertension *Two months ago blurred* vision and a feeling of slight confusion

Examination showed VOD 1 0 + 3 00 sph VOS 0 9 + 1 25 sph Motility and pupils were normal Ophthalmoscopy showed normal discs attenuated arteries of changing calibre no exudates Perimetry revealed a left homonymous hemianopia with sparing of the upper half of the macula in the left eye in the right the findings *above the* fixation point were too inaccurate for plotting

Right carotid angiography revealed a stenosis of the extracranial part of the internal carotid artery with decreased flow velocity No filling of posterior cerebral artery EEG showed slight to moderate change in the right temporal region ^{99m}Tc brain scanning showed nothing abnormal Clinical neurological examination showed nothing *abnormal*

Case 10 (130103) 69 year old man previously in good health who 6 months prior to admission experienced severe dizziness dim vision and a transitory right hemiparesis *The following* day a right hemianopia was observed

Examination showed visual acuity 0 6/ in both eyes with correcting glasses Motility and pupils were normal Ophthalmoscopy showed normal discs arteries of slightly uneven calibre with a v compressions The veins were normal Normal macular regions no exudates Perimetry revealed a right hemianopia with sparing of the lower half of the macula and some function on the right side of the hemiopic border below the fixation point

Left carotid angiography showed arteriosclerotic vessels EEG slightly abnormal with focal changes in the left temporo occipital region ^{99m}Tc scanning was normal Blood pressure 130/80

Case 27 (071003) 67 year old man in good health who had 1 year previously received trauma to the neck He was briefly unconscious and since then has complained of failing vision

Examination showed VOD 1 00–4 00 sph VOS 0 67–3 50 sph Motility and pupils were normal Ophthalmoscopy revealed normal discs with a slight peripapillar myopic halo The arteries were attenuated and of changing calibre Veins were normal There were slight macular pigmentary derangements Perimetry showed a complete left sided hemianopia with sparing of the lower half of the macula In addition there was a partial right sided hemianopia

Right carotid angiography showed arteriosclerosis of the vessels There was no filling of the posterior cerebral artery the other large vessels were normally situated ^{99m}Tc brain scanning revealed nothing abnormal EEG was normal Clinical neurological examination was normal except for a left sided dysdiadochokinesis and abolished achilles reflexes Blood pressure was 210/150

Case 43 (061103) 64 year old woman during the past year treated for arterial hypertension The last 3 weeks before admission she had noted failing memory and a sleeping sensation in the right sided extremities

Examination showed visual acuity 1 0 in both eyes with correcting glasses Motility and pupils were normal Ophthalmoscopy was normal Perimetry revealed a right homonymous hemianopia with sparing of the lower half of the macula

Left carotid angiography showed fairly pronounced arteriosclerotic vessels with reduced flow velocity No filling of left posterior cerebral artery Ventriculography showed some dilatation of the ventricles no signs of tumour Left vertebral angiography revealed no filling of the left posterior cerebral artery and a general arteriosclerosis of the vessels ^{99m}Tc scanning showed nothing abnormal EEG moderate diffusely abnormal Clinical neurological examination showed left dysdiadochokinesis right dominance of tendon reflexes on the arm No ataxia

Case 19 (281911) 61 year old man with arterial hypertension known for 10 years One month before admission he had had a brief attack with a feeling of unreality and distance and gyratoric dizziness Afterwards there was a slight right hemiparesis and hemianopia

Examination showed VOD 0 7 VOS 0 8 Eye movements and pupils were normal Ophthalmoscopy showed normal discs attenuated arteries with a v compressions Perimetry disclosed a right hemianopia with sparing of the lower half of the macula

Left carotid angiography revealed arteriosclerosis with decreased flow velocity ^{99m}Tc scanning showed an accumulation of activity in the anterior part of the left occipital lobe. EEG showed no focal abnormalities Clinical neurological examinat on

showed a slight right sided facial palsy and exaggerated right tendon reflexes The diagnosis was that of a thrombosis of the left posterior cerebral artery Vertebral angiography was not undertaken

Case 2 (170615) 56 year old woman admitted because of difficulties with seeing things in the right half of the visual field

Examination showed visual acuity in both eyes 1 0 + 0 50 sph Eye movements and pupils normal Ophthalmoscopy showed normal discs tortuous arteries of slightly changing calibre Veins were normal normal macular regions Perimetry showed a right homonymous hemianopia with sparing of the lower half of the macula

Left carotid angiography showed general arteriosclerosis Left vertebral angiography showed nothing abnormal Pneumoencephalography revealed a slight cortical atrophy otherwise nothing abnormal ^{99m}Tc brain scanning showed nothing abnormal Echoencephalography normal EEG normal Clinical neurological examination was normal except for a weak left achilles reflex

Case 12 (030742) 28 year old woman who developed left hemianopia with reading difficulties

Examinations showed visual acuity 0 9–1 00 sph in each eye Ophthalmoscopy was normal perimetry revealed a left hemianopia with sparing of the upper half of the macula

Right carotid angiography showed a forward displacement of the posterior branches of the medial cerebral artery suggestive of a tumour posteriorly in the right hemisphere. At operation a tumour was found and the occipital lobe was resected Microscopic examination showed a chronic granuloma

Case 2? (300741) 27 year old man with epileptic seizures (grand mal) since the age of 7–8 years treated with antiepileptics The patient is mentally retarded and at present complains of headache dizziness and spastic paresis of left extremities

Examination showed visual acuity 1 0 in each eye Right pupil larger than the left. Ophthalmoscopy showed hypoplastic slightly grey discs Perimetry showed right hemianopia with sparing of the macula only of the lower half on the left side

Carotid angiography (right and left) showed a right sided parasaggital tumour which on operation was found to be a meningioma of the falx

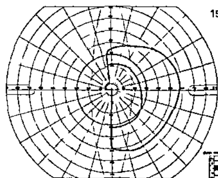
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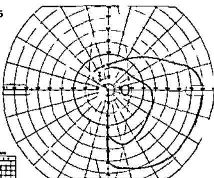
The reported cases illustrate that the sparing of the fixation area can take the form of a quadrant The patients cooperated well with visual field examination and in all cases the quadrant sparing could be demonstrated by campimetri as well as with the Goldmann perimeter The quadrantal shape which was often

Fig 1

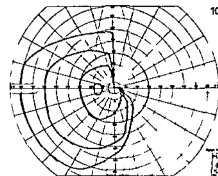
Visual fields in case nos 15 10 27 and 43

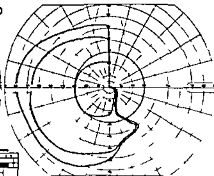
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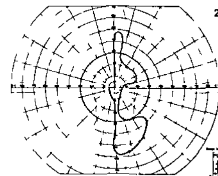


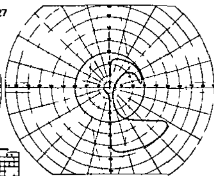
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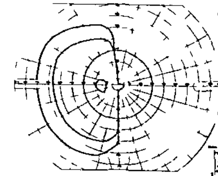


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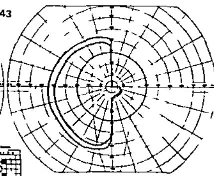


Table I
Data for eight patients with quadrant sparing of the macula

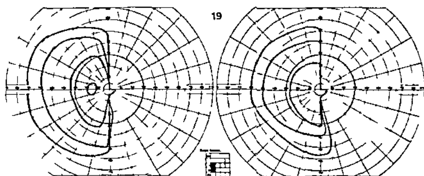
No	Age/sex	Hemianopia r/l	Sparing upper/lower	Neurological diagnosis
15	ι3/m	l	u	Arterial hypertension circulatory insuff no filling of p c.a
10	69/m	r	l	Arteriosclerosis EEG focus temporooccip
27	67/m	l	l	Arterial hypertension circulatory insuff no filling of p c a
43	64/f	r	l	Arterial hypertension Arteriosclerosis no filling of p c a
19	61/m	r	l	Thrombosis of left p c a Arterial hypertension
2	56/f	r	l	Circulatory insuff Arteriosclerosis
12	28/f	l	u	Granuloma of occipital lobe
22	27/m	r	l	Falx men ngioma

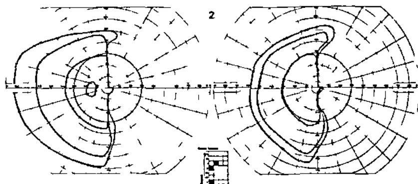
quite evident makes it improbable that the findings can be explained by a shift of fixation in the vertical direction

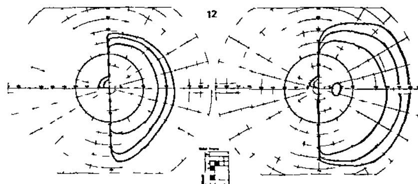
Data for the eight patients are summarized in the Table and the visual field findings in Figs 1 and 2 The series comprised three woman and five men with ages ranging from 27 to ι3 years In the two young patients the lesion was a tumour in the remaining six cases the lesion was a vascular one either due to arterial hypertension and/or arteriosclerosis

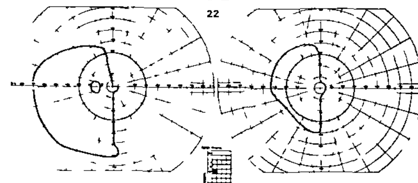
In analysing the case records *it is disappointing how little can be said about* the exact localization of the interruption of the optic fibres An arteriographically demonstrated occlusion of the posterior cerebral artery does not prove

Fig 2
Visual fields in case nos 19 2 12 and 22


19


2


12


22

whether it is the optic radiation or the cortex that is damaged When no filling defect of the posterior cerebral artery or its main branches could be demonstrated it might tentatively be suggested that the lesion lies in the smaller vessels in the cortex From the case histories it would appear that the lesion is in all cases *post geniculate*

The upper quadrant was spared in two cases and the lower one in six cases. A right hemianopia was present in five cases a left one in three cases

Numerous theories have been proposed to explain the macular sparing as set out in the introduction to this paper A complete discussion of the problems concerned with the sparing is not relevant here and can be found in several common textbooks It is sufficient to say that there is of course no reason to assume the same explanation in all cases of sparing The structured shape of the spared area in the reported cases points to an anatomical cause and virtually rules out the causes 4 5 and 8 in the introduction

The visual cortex receives its vascular supply almost entirely from the posterior cerebral artery (Beauvieux & Ristich Goelmino 1926 Kleihues 1966 Kleihues & Hizawa 1966 Mumenthaler 1973 Niesel 1973) but at least in some *cases part of the occipital pole is supplied by the middle cerebral artery* From an anatomical study of 32 human brains Smith & Richardson (1966) related the area of distribution of each branch supplying the visual cortex to the visual

Fig 3

The portions of the right half of the visual field projecting to visual areas supplied by the indicated arteries Redrawn from Smith & Richardson (1966)

field A simplified scheme of their findings is shown in Fig 5 The most interesting in the present connection is the horizontal border in the central field between the area supplied by the posterior temporal artery a branch from the posterior cerebral artery and the area supplied by the middle cerebral artery Therefore a simple vascular explanation would seem to apply in the six cases (Nos 10 27 43 19 2 and 22) in which the sparing was of the lower quadrant Sparing of the upper quadrant would require another course of the vessels Upper or lower quadrant sparing could not be correlated to any radiologically demonstrable occlusion of the posterior cerebral artery but it has to be remembered that filling of this artery is very variable due to its individually variable origin and to variable haemodynamic conditions during the angiography Anatomical studies comprising larger series have not been published but as emphasized by Smith & Richardson considerable variations occur in the distribution of the vessels

The reported cases document the existence of a quadrant sparing It would seem possible to conclude that the sparing in these cases is of vascular origin An anatomical explanation based on a horizontal separation of the nerve fibres is also a possibility but to the knowledge of the author no such anatomical substrate has been demonstrated As regards the clinical implications a quadrant sparing therefore suggests a vascular lesion localized to the cortex or subcortex

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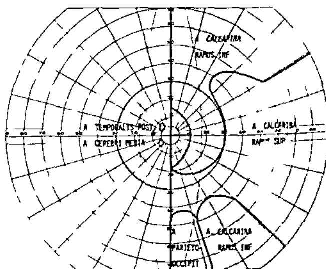


Fig 3

The portions of the right half of the visual field projecting to visual areas supplied by the indicated arteries Redrawn from *Smith & Richardson (1966)*

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University of Illinois Eye and Ear Infirmary
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HISTOCOMPATIBILITY MATCHING IN POOR PROGNOSIS PENETRATING KERATOPLASTY

BY

THOMAS O BENNETT GHOLAM A PEYMAN
ROBERT TISSOT and CARL COHEN

Rabbits were haplotyped for the major rabbit leukocyte locus A (RL A locus) which is analogous to the human leukocyte locus A (HL A locus) The rabbits were divided into five groups and the groups were arranged to provide a controlled experimental setting Standardized alkali burns were induced in selected groups to produce heavy vascularization of the corneal bed

Groups with completely histocompatible corneal donors for the RL A locus had a 9 out of 10 success rate when keratoplasty was performed on a vascularized bed Another group of rabbits mismatched at the RL A locus with histoincompatible corneal donors were prepared in the same manner and had a success rate of 1 out of 9 A probable significant difference ($P < 0.005$) using Fischer s exact test was found between the two groups

Key words alkali burns – histocompatible – HL A locus – RL A locus – corneal vascularization – sensitization – penetrating keratoplasty – autologous

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patible (Ehlers & Ahrons 1971) Thus it appears that an immune process is capable of being elicited in keratoplasty

However histocompatibility matching in keratoplasty has not been found to be of any clear advantage in clinical studies (Gibbs et al 1973 Allansmith et al 1974) The present study was initiated in an attempt to re evaluate this controversy We desired to observe the effects of histocompatibility matching and mismatching at the rabbit leukocyte locus A (RL A) in performing keratoplasty into vascularized corneal beds We created a controlled setting in which immunologic reaction would be enhanced in order to determine the efficacy of histocompatibility matching

Material and Methods

Animals

The rabbits came from an essentially closed colony that was maintained to study serologically detected polymorphic systems The RL A locus which is analogous to the HL A in humans was identified by the cytotoxicity technique of Tissot & Cohen (Tissot & Cohen 1972)

Alkali burns

In order to induce a uniform area of corneal vascularization alkali burns were produced randomly in either the right or left eye of each rabbit in three groups Each animal was first anesthetized with 3 ml of pentobarbital sodium in a 1 3 dilution with sterile normal saline via an ear vein The eye was proptosed and stabilized with toothed forceps Two drops of proparacaine hydrochloride (Ophthaine) were applied topically One drop of a 1 0N sodium hydroxide solution pH 11.5 was placed on each side of a 12 mm lamella of micro filter paper which was then applied to one of the rabbit s corneas for 40 sec The limbus was avoided in each case Immediately after removal of the lamella the eye was rinsed with distilled water for 60 sec Neosporin ointment was applied topically on each burned eye once a day for 5 days

Procedure for penetrating keratoplasty

The side of each rabbit s head corresponding to the burned cornea was shaved and prepared for surgery Anesthesia was induced by a 3.5 ml injection of a 1 3 pentobarbital sodium sterile normal saline solution via an ear vein Each animal received 0 1 ml of atropine sulfate intramuscularly to dry respiratory secretions

A lateral canthotomy was performed The eye was then proptosed and the conjunctiva dissected away from the sclera and extraocular muscles The superior and inferior rectus muscles were stabilized using a scleral open ring (Fig 1) which was sutured into place using 6–0 catgut The scleral open ring with its handle allowed excellent control of the eye when needed at the time of trephination The ring was left free during surgery as a safety measure for control of the globe in case the eye retracted before the completion of surgery

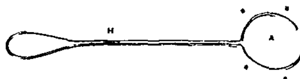


Fig 1

Scleral open ring which allowed stabilization of eye during keratoplasty procedure. Handle (H) allows surgeon easy control of eye for trephination Sclera of eye was sutured to arms of ring (arrows) and cornea projected anteriorly (A)

An intravenous injection of 1 0 ml of aqueous heparin sodium 1 1 000 via an ear vein prevented aqueous clotting At the time of trephination 1 1 000 aqueous heparin sodium was applied topically to each eye by continuous drip as soon as the anterior chamber was entered This was followed by instillation of a few drops of heparin sodium every 2 to 4 min during the course of surgery

Donor and recipient rabbits were both prepared in this manner Transplantation was performed on the eyes that had received an alkali burn Each donor provided two corneas one for each recipient Three rabbits two recipients and the donor made up a donor recipient triad Donor corneas were randomly taken from either the right or left eye A 7 0 mm Castroviejo trephine was used for the initial incision after one drop of sterile fluorescein was applied to allow visualization of the incision line The circular incision was completed with fine, curved scissors

The procedure for the initial incision in the recipient was essentially the same as that used for the donor This was done using a Zeiss operating microscope \16 The corneal button was placed on the recipient s eye four 9–0 Ethilon sutures in each of four quadrants stabilized the graft and a running suture secured it

Composition of experimental groups

Group 1 Six rabbits received an alkali burn in one eye They served as burn controls for 6 months after which they were killed by intravenous injection of pure pentobarbital sodium

Group 2 The six rabbits in this group received autogenous keratoplasty in one eye but their corneas were not burned prior to transplantation These served as controls for technique.

Group 3 This group of rabbits did not receive corneal burns and their corneas were transparent at the time of grafting They received donor buttons from mismatched histoincompatible rabbits Each donor contributed two corneas one to each recipient. This made a donor recipient triad There were three donor recipient triads in this group for a total of nine rabbits Donor tissue was provided from either the right or left eye in a random fashion throughout the study

Group 4 These rabbits each had one eye burned 6 weeks before surgery Each eye showed extensive vascularization of the cornea The donor tissue came from mismatched or histoincompatible rabbits This group allowed a direct comparison of results between mismatched and matched poor prognosis corneal grafts There were five donor re cipient triads in this group or 15 rabbits i e 5 donors and 10 recipients

Group 5 These rabbits also received corneal burns 6 weeks before surgery In this case each rabbit was matched completely at the RL A locus There were five donor and recipient triads for a total of 15 rabbits in this group

Observation and histologic evaluation

Postoperatively each animal was kept in a separate cage and received Neosporin oint ment topically for 3 days The rabbits were examined with a slit lamp daily for the first 3 weeks then every third day for 2 1/2 months The observer and surgeon were unaware of which rabbits belonged within the various classifications Sutures were re moved 14 days after surgery Schiotz tonometers were used to record intraocular pressure at the time of surgery at suture removal and the end of the study

Rabbits in group 1 were killed 6 months after production of corneal burns Those in groups 2 3 and 5 were killed 3 months after keratoplasty by means of intravenous pentobarbital Those in group 4 were sacrificed at various periods depending upon the onset of graft rejection The eyes were enucleated and the corneas were fixed in a 1 1 solution of formaldehyde glutaraldehyde dehydrated with ethyl alcohol embedded in paraffin and stained with hematoxylin and eosin for histologic study

Results

Graft survival by group

Keratoplasties were not performed in group 1 However in each case alkali burns of the cornea produced an opaque cornea with severe vascularization that remained during the entire 6 month period of study

Ninety days was set as the terminal point for declaring the transplant either a success or failure due to the fact that most graft reactions are encountered in the first 3 months in both kidney and corneal transplants A graft was considered successful when it remained entirely clear (Fig 2) The beginning of vascular ingrowth graft opacification rejection line or development of anterior synechiae were noted Onset of graft reactions determined the end point of graft survival An immune response was noted if (a) no pre or post operative complications were encountered (b) the graft was clear for 2 weeks prior to graft reaction (c) the reaction was characterized by mild edema of the cornea (d) the presence of keratic precipitates was considered diagnostic of immune rejection

In group 2 transplants with clear autologous tissue all corneas (100 %) re mained transparent for the entire 90 day period (Table I) No complications



Fig 2

Rabbit eye that received histocompatible corneal tissue after keratoplasty Cornea is clear with no signs of scarring vascularization or rejection

Table 1

Corneal transplant data – 7 mm graft survival in days

Data chart shows *graft survival in days for each transplant with* 90 days as the terminal point of the experiment

Good prognosis groups		Poor prognosis groups	
Group 2 Autologous no burn	Group 3 Mismatched no burn	Group 4 Mismatched NaOH burn	Group 5 NaOH burn Matched
1 90 days	1 90	1 90	1 90
2 90	2 90	2 39	2 90
3 90	3 90	3 34	3 90
4 90	4 90	4 3[illegible]	4 90
5 90	5 61	5 32	5 90
6 90	6 56	6 28	6 90
90 0	6 1*	7 25	90
		8 23	8 90
		9 21	9 90
		36 0*	10 56
			86 6

* Mean graft survival

were encountered and intraocular pressures remained within normal limits Interestingly circumcorneal injection occurred in each case intensifying with time By the ninth day a clear vascular ingrowth toward the suture line could be detected after surgery However in each case this response subsided with the removal of the sutures and the graft remained viable

Group 3 rabbits receiving clear mismatched grafts had an average graft survival of 76 1 days with four of six (66 6 %) corneas remaining clear for the entire 90 day period The two grafts that failed developed anterior synechiae in the lower quadrant of the graft This caused increased intraocular pressure and buphthalmos

Group 4 with heavily vascularized mismatched corneal beds after alkali burns showed rejection in 8 of 9 grafts One graft remained clear throughout the experiment One rabbit died 3 days after surgery Thus nine rabbits made up this group Surgical complications were avoided and the mean graft survival time was 36 0 days

In group 5 histocompatible matches were achieved in severely burned corneas and 9 of 10 grafts remained clear for the duration of the experiment In the one case of failure the rabbit developed a hyphema from iris trauma at surgery This anterior chamber hemorrhage appeared to be resolving when another episode of trauma in handling the rabbit caused a second hemorrhage Opacification followed this incident The average graft survival in this group was 86 6 days



Fig 3
Development of tuft like even vascular ingrowth from limbus (arrows) after alkali burn. The central cornea is completely opaque



Fig 6
Chronic rejection at 3 months after transplantation The limbal vascular ingrowth is obvious (arrows) and the central cornea shows vessel invasion with scarred cornea (S)

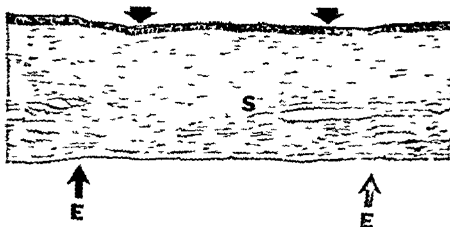


Fig
Cornea from histocompatible group 3 that remained clear througho ut the study The epithelium (top arrows) is intact with normal cellular configurat n The stroma (S) shows normal histological structure with keratocytes present Absen e of leuk cytes and vascular proliferation is obvious Endothelium (E arrows) i ntact (100



Fig 8

Acute reaction in poor prognosis group Massive stromal hypercellularity vascular in growth (arrows) and perivascular leukocyte cupping with leukocyte infiltration through out the stroma Response is mainly anterior (A) with some infiltration to posterior stroma (P) (x 100)

Histology

Each eye in group 1 showed a diffuse infiltration of fibrocytes distinct stromal vascularization and scarring

Preparations of eyes from groups 2 3 4 and 5 that remained transparent after keratoplasty showed normal corneal histologic relationships (Fig 7) with intact epithelium stroma and endothelium Vascularization and edema of the graft were not encountered

Graft reactions began at the periphery of the donor host interface As vascular invasion from the donor reached the recipient scar lymphocytes monocytes and plasma cells infiltrated the surrounding tissue (Fig 9) These vessels were pre dominantly anterior in their approach to the stroma Secondary stromal edema resulted This infiltration eventually reached the endothelial cells which were destroyed in this process This chronic process gave an eventual picture of scarred corneal tissue

Discussion

The primary goal of this study was to evaluate the effects of histocompatibility matching in the poor prognosis group of severely vascularized corneas Immunologic graft rejection is caused by a combination of cellular and humoral immunity which is in turn caused by a number of factors including the presence of strong and weak histocompatibility antigens in the donor tissue and their absence in the recipient tissue One phase of rejection seems to be determined by both the number and strength of the histocompatible antigens within the graft Strong and weak antigens exist however little is known about them except that weak antigenic incompatibilities can be overcome with immunosuppression while stronger systems such as the HL A loci should be matched In this study immunosuppression was avoided but RL A matching or mismatching was accomplished

The HL A system is determined by two segregated series of alleles belonging to two linked loci called LA and Four These alleles are apparently codominant, and the corresponding antigens act analogously Thus each human should have at most four different HL A genes and corresponding antigens An A match indicates that the donor and recipient have the same HL A identity Group 5 contained rabbits in this classification A G match indicates absence of any common HL A antigens by donor and recipient Animals in all other groups of the study fit this designation Thus all rabbits had identified major RL A loci which were either matched or mismatched however minor antigenic loci were not matched

In severely vascularized corneas A matched histocompatible donor recipient triads produced a significant graft survival (86 6 days 9 10 success rate) approaching that of autologous clear transplants (90 0 days 100% success rate) These histocompatible transplants were more successful than the mismatched or incompatible group receiving alkali burns and keratoplasty (36 0 days 1 9 success rate) (Table I) In this study matching histocompatibility antigens affected the poor prognosis group of transplants and is probably of significance by Fischer s exact test ($P < 0\ 005$) Clinical studies have failed to show histocompatibility matching of significance in keratoplasty (Gibbs et al 1973 Allansmith et al 1974) However control in such clinical situations is almost impossible to obtain and the severely vascularized recipient bed with a bad prognosis was sparsely represented in early studies In addition the use of immunosuppressive agents only clouds the true issue of graft rejection in such clinical settings In animal studies these problems can be avoided This study was conducted in a controlled manner and a standardized technique was used to induce vascularization of the corneal bed Immunosuppression was not employed

The class of keratoplasties with severe chemical burns and extensive vascularization of the corneal bed has generally been accepted as the poor prognosis group with a low success rate (Polack 1973) The rejection process is related to the sensitization of the host to donor tissue and may be brought about by vascularization of the graft bed with the production of lymphatic vessels i e the afferent arc of the immunologic response In earlier work lymphatics have been found in experimentally vascularized rabbit corneas (Collin 1966 Smolin 1971 Smolin et al 1972 Polack 1966 and Polack & Gonzales 1968) This could explain the initiation of rejection in vascularized corneal beds

In this regard each rabbit in groups 4 and 5 had a graft bed extensively vascularized by a standardized technique at the time of keratoplasty The 6 weeks following burn applications had allowed time for tissue sensitization to occur and provided a ready access for vascular infiltration at the corneal button host tissue interface The role of vascularization in the production of sensitization is one explanation for the poor prognosis encountered in cases where a vascularized graft bed exists prior to transplantation The difference between matched and mismatched grafts in this study strongly suggests the efficacy of histocompatibility matching in the face of such sensitization of the host

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THE DIAGNOSIS OF CHRONIC SIMPLE CONJUNCTIVITIS

Vital Staining of Tarsus with Tetrazolium – Alcian Blue Mixture

BY

M S NORN

Vital staining with a tetrazolium – alcian blue mixture gave a characteristic punctate red staining of the superior or the inferior tarsus in 69 % of all examined patients with chronic simple conjunctivitis (69 patients subjected to 168 examinations) The superior tarsus was more frequently stained than the inferior (61 % against 33 %) The staining was most often concentrated centrally or in the middle anterior part of the superior tarsus If located on the inferior tarsus it was most often found medially anteriorly No corresponding staining was seen in normal eyes The differential diagnosis involving keratoconjunctivitis sicca and pemphigoid is mentioned The staining is due to enzymatic reduction of tetrazolium in the cytoplasm of the tarsal epithelial cells The positive vital staining finding is consistent with a diagnosis of chronic simple conjunctivitis

Key words conjunctivitis – chronic simple conjunctivitis – diagnosis – vital staining – tetrazolium – tetrazolium alcian blue mixture

Chronic simple conjunctivitis is characterized by conjunctival complaints (smarting pain itching grain of sand sensation) lasting several months without

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associated physical signs The disease is refractory to treatment (antibiotics antihistaminics and mucomimetics are ineffective Astringents have varying transitory effects Such mild agents as 0 9 % sodium chloride or 2 % sodium bicarbonate often have the best effect)

The diagnosis is one of exclusion other causes of similar complaints have to be excluded (refraction anomalies inadequate convergence etc)

It would be an important aid if a physical sign characteristic of chronic simple conjunctivitis could be detected partly to enable a diagnosis to be made and partly to provide a chance of disclosing its cause

The results achieved by histological cytological bacteriological and sensitivity studies are the same both in patients suffering from the disease and in normal subjects The conjunctival mucous thread is as mobile as in normal eyes and the quantity of mucus is barely increased The tear production and its pH are normal Vital staining by fluorescein rose bengal trypan blue methylene blue bromothymol blue lissamine green etc corresponds to that in normal eyes (Norn 1974) The wetting time (break up time) of the precorneal film is normal (Norn 1969)

The amount of foam in the outer canthus is often augmented (Norn 1963) However this increase in the amount of foam is also seen in other diseases (foreign body on cornea painful intrabulbar affection) Furthermore foam often occurs in the eyes of young normal individuals The increased amount of foam may be due to frequent or intense blinking The finding in other words is not specific for simple chronic conjunctivitis

On vital staining with tetrazolium *alone* I noticed a significantly increased staining of the inferior tarsus in chronic conjunctivitis as compared with normal eyes (in 36 % of 44 eyes with simple conjunctivitis against 14 % of 59 normal eyes $P < 0\ 02$ Norn 1971)

On vital staining with a *mixture* of tetrazolium and alcian blue I found a characteristic punctate tetrazolium staining of the tarsus in simple conjunctivitis (Norn 1972 a)

The object of the present study was partly to obtain if possible a verification of this finding in a larger series of simple conjunctivitis cases and partly to follow the individual patient to see whether the stainability alters in the course of weeks or months

Method

The patient was vital stained by a tetrazolium – alcian blue mixture composed as follows

iodonitrotetrazolium	100 0 mg
alcian blue	250 mg
phenyl mercuric nitrate	0 1 mg
distilled water to	10 0 g
aseptically prepared	

A small drop (0 01 ml) of vital stain mixture is instilled on the lateral part of the bulbar conjunctiva while the patient looks down and nasally Contingent tetrazolium staining at the point of contact may be artificial Staining at this site is therefore disregarded

The vital staining is estimated in the slit lamp not less than 4 min after instillation The time of reading after the 4–min interval is not critical because the tetrazolium staining will last for several hours (Norn 1972 b)

Punctate red staining represents a special form of degenerative live cells The colourless substance tetrazolium penetrates into the cell where within a few minutes it becomes enzymatically reduced to the red dye known as formazan

Blue staining indicates alcian blue stained conjunctival mucus

The staining is estimated in white slit lamp light In doubtful cases reading is also performed in red free light (green filter) which permits an easier distinction between vessels and superficially located red vital stained dots

The staining intensities are graded from 1 to 5 Four indicates intense staining, 2 weak 3 moderate and 1 and 5 are the extremes The grade and site of the staining are entered in a diagram for each eye.

Material

The investigation comprised 69 patients with typical chronic simple conjunctivitis which had troubled them for months or more often for years They had been treated with different drugs with little or no effect Some of the patients came from my own practice (Vanlose) and the others from the Out Patient Ophthalmic Clinic Kommunehospitalet

Thirty six patients had their eyes controlled and vital stained repeatedly at intervals ranging from weeks to months These patients were examined on an average 3 75 times each

The total material comprised 168 examinations of altogether 336 eyes (672 tarsi) in 69 patients

Result

Out of 23 *normal* eyes none showed tetrazolium vital staining of the superior or the inferior tarsus after instillation of the dye mixture used for the present study (Norn 1972 a) On the other hand in the series of single chronic conjunc

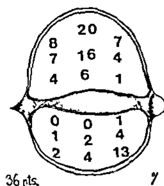


Fig 1

Sites of tetrazolium staining of tarsi in chronic simple conjunctivitis A percentage calculation of sites in 36 patients after staining with a tetrazolium – alcian blue mixture

tivitis under review the incidence of tetrazolium staining was high It may be expressed as incidence per *individual* per *eye* or per *region* (superior or inferior tarsus)

Staining of the tarsus was seen in 69 % of all the *patients* (not less than one tarsus of at least one eye) There was no significant difference between the incidence at the primary examination (66 7 %) and the incidence at the sub sequent follow up examinations (70 7 %)

Staining most frequently involved the superior tarsus at least one superior tarsus was stained in 61 % of the subjects as compared with the inferior tarsus in only 33 % of the patients ($t = 5\ 4$ $P \ll 0\ 001$)

Tarsus staining (superior and/or inferior) was seen in 51 % of all the eyes There was no difference between right and left eyes

The superior tarsus was stained in 43 % of the eyes and the inferior in 23 % The difference from the normal series was significant ($P < 0\ 001$ and $P < 0\ 05$)

Site

Tetrazolium staining occurred more often on the superior tarsus than on the inferior On the superior tarsus it was most frequently seen in the centre or anteriorly in the middle always definitely behind Marx line On the inferior tarsus it was most frequently localized medially and anteriorly behind Marx line Fig 1 illustrates the percentage distribution of sites in 36 patients Often two or more regions were stained at the same time e g four or five horizontal

lines on the superior tarsus anteriorly or centrally or a band of stained dots behind Marx line in the middle or medially on the inferior tarsus Staining posteriorly on the inferior tarsus (at the fornix) was extremely rare

The staining might be conceived to bear relation to the point of contact of the dye with the bulbar conjunctiva where artificial staining may be seen However in only 11 out of 36 eyes were the tarsus and the eyeball stained at approximately corresponding sites In 17 eyes no artificial staining of the eye ball was seen despite intense staining of the tarsus

The tetrazolium staining manifested itself by many fine red dots often grouped in long lines running a horizontal course (parallel with the lid margin) more rarely in an oblique or vertical course They may be collected in large stained groups Mucus was seldom seen in relation to the red dots

Fig 2 illustrates the profile of the tetrazolium – alcian blue vital staining in chronic simple conjunctivitis The mean staining grade has been stated for the different localities It is seen that tetrazolium staining of the inferior tarsus and particularly of the superior tarsus is characteristic of chronic simple conjunctivitis whereas dots of mucus are scarce in these regions

The *cornea* was rarely stained but if so was only pale in colour The *bulbar conjunctiva* showed moderate tetrazolium staining and staining of mucus but always as faint red and blue dots usually localized infero nasally or nasally as may also be seen in normal eyes In no instance was concurrent staining observed of the cornea and the exposed part of the bulbar conjunctiva In other

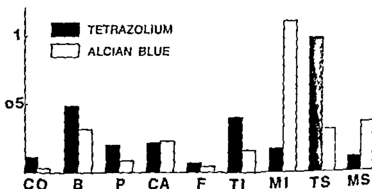


Fig 2

The profile of tetrazolium – alcian blue vital staining in chronic simple conjunctivitis *Ordinate* Mean staining grade at site concerned (The colour intensity for each patient was graded arbitrarily in grades from 1 to 5) *Abscissa* Site proceeding from the 0 point Cornea eyeball plica caruncle inferior fornix inferior tarsus Marx line of lower lid superior tarsus and Marx line of upper lid.

words the staining was never reminiscent of that in cases of keratoconjunctivitis sicca (cf Bijsterveld 1969)

Plica and caruncle were stained as in normal eyes The fornix was practically never stained

Marx line of the inferior lid was found to be alcian blue – stained (mucus) in more than half of the cases but only 11 % showed slight tetrazolium staining

In Marx line of the superior lid mucus staining likewise predominated over tetrazolium staining

Table I shows the percentage of eyes stained by tetrazolium or alcian blue in the region concerned

The mucous thread in the inferior fornix was normal with no signs of pre dominance of inflammatory cells (The blue stained mucous regions were larger than the red stained regions)

Repeated examinations

Thirty six patients were examined repeatedly at intervals ranging from weeks to months

Fig 3 illustrates four examples

I M showed no tetrazolium staining on the first examination On the second both superior tarsi were stained characteristic of chronic simple conjunctivitis The last three examinations revealed no staining

Table I

Tetrazolium – alcian blue staining in chronic simple conjunctivitis The figures represent the percentage numbers of eyes stained in the region concerned

	Tetrazolium	Alcian blue
Cornea	11 6	[illegible] 2
Bulbar conjunctiva	31 2	19 6
Plica	13 0	[illegible] 2
Caruncle	16 7	14 5
Inferior fornix	3 6	[illegible] 2
Inferior tarsus	[illegible] 9	11 6
Inferior Marx line	10 9	[illegible] 1
Superior tarsus	43 8	16 7
Superior Marx line	8 7	[illegible]6 1

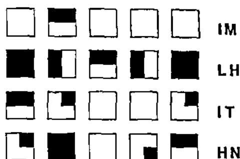


Fig 3

Tetrazolium staining of four patients tarsi each stained five times at intervals of from weeks to months Each square symbolizes all four tarsi It is seen that H N on first examination only showed staining of the left superior tarsus

L H had all four tarsi stained initially Twice both right tarsi were stained and on the last examination all four tarsi again

I T had the same site stained twice (left superior tarsus)

In H N the staining had different sites on the four occasions where staining occurred

In the total material identical sites were found in 19 out of 64 possible cases (30 %) though often of varying intensity

Microscopy

Microscopy of smears from tetrazolium stained tarsal mucosa showed numerous red granules in the cytoplasm of columnar and cuboid epithelial cells found scattered among unstained epithelial cells

No red staining was seen in neutrophilic leukocytes or in other cells of this smear

Discussion

A physical sign of the disease chronic simple conjunctivitis has now been demonstrated for the first time This sign is the punctate red vital staining of the superior and/or inferior tarsus in response to instillation of a tetrazolium - alcian blue mixture

The staining is because in the presence of this disease iodonitrotetrazolium is able to penetrate into the epithelial cells of the tarsus where *enzymes* in the cytoplasm can reduce the substance to red formazan

The phenomenon is unlikely to result from rubbing (no right sided predominance – particularly no increase in patients disposed to frequent rubbing)

Two factors are worth pointing out The phenomenon occurs most often on the superior tarsus and when present on the inferior tarsus it occurs mainly medially

The phenomenon is not due to reduced tear secretion the staining not being particularly concentrated on the exposed areas of the cornea and bulbar conjunctiva

It is improbable that the staining is due to a reduced mucus production as in pemphigoid as the mucous thread in the inferior fornix was of normal size

Mucus is deposited around sutures on dead cells and in relation to rose bengal stained degenerate epithelial cells Mucus is rarely seen in relation to tetrazolium stained cells on the tarsus in chronic simple conjunctivitis This also argues in favour of a special form of degeneration

Unfortunately the phenomenon is variable The site on the tarsus alters in the individual patient with stain free intervals These alterations seem to bear no definite relation to treatment or fluctuations of the subjective complaints

Two thirds of patients with chronic simple conjunctivitis can be expected to show punctate red staining of the tarsus on a single examination Such staining does not take place in normal eyes

Punctate red staining of the tarsus may however also be seen in keratoconjunctivitis sicca and pemphigoid but the *differential diagnosis* is not difficult In sicca the staining is most pronounced over exposed areas (cornea bulbar conjunctiva) in other words outside the tarsal region In pemphigoid the mucous thread in the inferior fornix is absent or rudimentary The bulbar conjunctiva and possibly also the cornea may show considerable vital staining and symblepharon bands are present (Kristensen et al 19/4)

The positive finding of red staining concentrated particularly on the tarsus after instillation of a tetrazolium – alcian blue mixture thus confirms a diagnosis of chronic simple conjunctivitis No conclusions can be drawn from a negative finding but repeated examinations may perhaps subsequently result in a positive vital staining finding in the suspected patient

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management has primarily consisted of anterior approach procedures (Chandler 1964 Shock et al 1973 Zagora 1970) which have led to further vitreous loss and intraocular manipulation

Preliminary use of the Peyman vitrophage (Peyman et al 1973 Peyman & Dodich 1971 Peyman & Huamonte) in ocular reconstruction excision of dense secondary membranes and cataract extraction has been encouraging (Peyman & Diamond Peyman & Goldberg Peyman & Swartz) We attempt to illustrate the wide range of therapeutic application of the vitrophage in 15 cases of intra ocular trauma.

Case Reports

Case 1

An 8 year old black boy was hit in the right eye in August 1974 He sought attention 3 days after trauma at the Illinois Eye and Ear Infirmary Initial examination revealed a visual acuity of light perception 3+ edema of the eyelids and 360° of subconjunctival hemorrhage. A hyphema obscured 90% of the anterior chamber with an accompanying intraocular pressure of 62 mmHg The patient was hospitalized and treated with intra venous mannitol and acetazolamide topical homatropine and dexamethasone The intra ocular pressure was controlled in 14 days to the 15 to 22 mmHg range A B scan ultra sonogram showed a normal posterior segment The traumatic hyphema slowly resolved

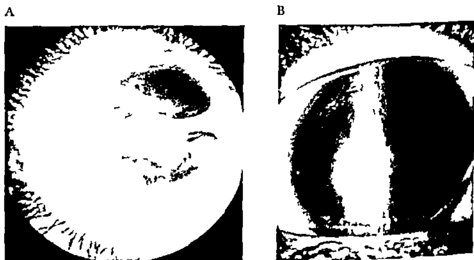


Fig 1

Case 1 A Preoperative view showing secondary membrane formation and pupillary occlusion. B Postoperative view Note wide pupillary opening Vitrophage has cut away occluding membranes

A



B



Fig 2

Case 2 A Preoperative slit lamp photograph showing dense cataract secondary to trauma *Iris adhesions prevented adequate dilatation* B *Postoperative view Vitrophage* has excised lens and cut iris adhesions Arrow indicates corneal scar

to a persistent fibrinous clot with a pupillary membrane adhering to partially resorbed lens (Fig 1 A) This dense fibrovascular membrane covered the pupil horizontally

Patient was readmitted on October 2? for membranectomy and vitrectomy Examination revealed visual acuity of light perception with four quadrant projection and positive color perception The fibrovascular pupillary membrane had further organized Eight degree exotropia was present The intraocular pressure was 10 mmHg and 4+ beam and 4+ cells were present in the anterior chamber

Immediately after surgery there was a 20/20 view of the fundus with a cup/disk ratio of 3 and a normal macula Postoperatively the intraocular pressure was elevated in the low 30 s The patient was treated four times a day with 125 mg of acetazolamide atropine sulfate 1 % and Maxitrol drops

By December 14 the corrected vision was 20/25 with +9.50 sph + 0.50 cyl x 90 A 12° right exotropia was present The cornea was clear (Fig 1 B) The intraocular pressure was still in the 30 s The patient was treated with 125 mg of acetazolamide four times a day atropine sulfate 1 % and Maxitrol drops three times a day and epinephrine hydrochloride 2 % twice a day Presently the untreated intraocular pressure is 21 mmHg

Case 2

In April 1974 a 9 year old white boy sustained a corneal laceration from a metal soft drink bottle cap involving the right lens The cornea was subsequently repaired. Over the next 6 months a dense pupillary membrane and cataract developed (Fig 2 A) Examination on November 19 revealed a visual acuity of hand motions at 2 ft the intraocular pressure was 1 mmHg The cataract prevented visualization of the fundus

The following day the patient underwent lensectomy membranectomy and anterior

vitrectomy via a pars plana approach On December 14 postoperative examination of the right eye revealed a visual acuity of 20/60 with +12.25 sph +1 00 cyl x 75° The corneal scar in the visual axis accounted for slightly decreased visual acuity (Fig 2 B)

Case 3

A 21 year old white man was hit in the right eye with a broomstick He sustained a nasal 26 mm corneoscleral laceration (16 mm corneal and 10 mm scleral) Iris prolapsed through the wound At that time his visual acuity was light perception The laceration was repaired that day Total hyphema subsequently developed Ultrasonography 5 days after operation demonstrated vitreous hemorrhage and nasal retinal detachment By the eighth day the lens had become cataractous

At 4 months postoperatively the patient was admitted for lensectomy and vitrectomy with the Peyman lens fragmenter and vitrophage Visual acuity at that time was hand motions He was able to perceive color and had two quadrant projection Immediately after surgery there was 20/20 visualization of the fundus An inferior nasal tractional retinal detachment was visible and the macula was on The retinal detachment was subsequently repaired with an encircling procedure and intravitreal gas injection

Postoperatively the patient had elevated intraocular pressure in the 30 s successfully controlled with acetazolamide Fourteen days after the retinal detachment repair the encircling band was loosened because of severe intraocular pain No further pain followed the procedure

Nine months postoperatively the visual acuity was 20/200 with +9 00 sph +2 00 cyl x 135° He had 20° exotropia There was a corneal scar nasally and preretinal traction membranes (Fig 3 A B) which help explain the decreased visual acuity The intraocular pressure was 16 mmHg The fundus was clear and the retina remained attached



Fig 3

Case 3 A Postoperative slit lamp photograph Note nasal corneal scar (arrow) B Post operative fundus photograph showing clear media Arrows denote preretinal traction membrane O indicates optic disk



Fig 4

Case 4 Postoperative fundus photograph showing white fibrotic scar inferior to disk Note displacement of the retina Arrows indicate optic disk

Case 4

A 30 year old white man was struck in the left eye on June 1? 1974 with a metal fragment The eye sustained a 7 mm corneoscleral laceration at / o clock (3 mm corneal and 4 mm scleral) There was lens perforation and vitreous hemorrhage The foreign body lodged in the posterior fundus adjacent to the retina The patient underwent repair of the corneoscleral laceration and removal of the metallic foreign body (5 to mm) with a magnet

Three weeks later the examination of the left eye revealed a visual acuity of light perception with no projection The conjunctiva had 3+ injection the anterior chamber displayed ?+ beam and 2+ cells the intraocular pressure was 15 mmHg Evidence of posterior subcapsular changes were becoming apparent The vitreous was full of blood On July 9 the patient underwent lensectomy and vitrectomy using a pars plana approach with the Peyman lens fragmenter and vitrophage At the time of surgery there was a 20/20 view of the posterior pole, revealing the site of the penetrating foreign body 2 disk diameters inferior to the disk

Postoperative examination of the left eye on December 13 revealed visual acuity of hand motions at 3 ft with a +11.50 lens The cornea showed a 3 mm scar at o clock. The intraocular pressure was 30 mmHg Retinal examination revealed white fibrotic scar 2 disk diameters inferior to the disk (Fig 4) with radiating preretinal traction and displacement of the macula The intraocular pressure was controlled with pilocarpine ?% four times a day

Cases 5 through 15 along with cases 1 through 4 are summarized in Table I

Table I
Results of vitrectomy to treat trauma

Case Sex Age (yrs)	Traumatic conditions and surgery preceding vitrectomy and membranectomy	Visual acuity	
		Preoperative	Postoperative
1 M 8	Blunt trauma hyphema increased intraocular pressure hyphema resolved with persistent fibrin clot and pupillary membrane	LP	20/25
2 M 9	Blunt trauma corneal laceration involving lens primary repair of cornea lens resorbed leaving pupillary membranes	HM	20/60
3 M 21	Blunt trauma corneoscleral laceration vitreous hemorrhage and nasal retinal detachment *uveal prolapse primary repair of laceration*	HM 2 ft	20/200
4 M 30	Intraocular foreign body corneoscleral laceration with vitreous hemorrhage metal extracted with magnet	LP	HM 3 ft*
5 M 10	Nail in eye corneoscleral limbus laceration with uveal prolapse diffuse hyphema repair of laceration and excision of prolaps returned 2 yrs later with updrawn pupil and vascular fibrous membrane	20/300	20/40
6 M 18	BB pellet hit eye resulting in hyphema iritis iridodialysis lens rupture and cataract discission and aspiration with vitreous loss sector iridectomy	20/400	20/40
7 M [illegible]	Blunt trauma dislocated lens vitreous hemorrhage retinal d[illegible] [illegible] angle recession	1/200	20/50
8 M 7	Blunt [illegible] primary repair of [illegible]ornea 1 yr ago [illegible]	LP	20/[illegible] [illegible]**

9 M 64	Blunt trauma dislocated lens corneal edema	HM 3 ft	20/400
10 M 34	Intraocular foreign body perforated lens metal extracted with a magnet irrigation of lens secondary lenticular membrane vitreous hemorrhage	I P	HM 2 ft*
11 M 14	Blasting cap explosion corneal laceration involving lens vitreous hemorrhage retinal injury	2/200	20/70
12 F 51	Blunt trauma corneoscleral laceration uveal prolapse vitreous hemorrhage primary repair of laceration uveal excision postoperative hyphema glaucoma posterior synechia lens swelling	LP	20/300
13 M 15	Blunt trauma corneal laceration iris prolapse ruptured lens repair of laceration and excision of iris prolapse much lens material remained	HM 6 in	20/50
14 M 20	Penetrating pencil injury corneal laceration involving lens vitreous hemorrhage cataract extraction (1968) 7 yrs after trauma retinal detachment occurred 6 yrs later pupillary membranes observable	HM 3 ft	20/400
15 M 14	Penetrating copper blasting cap penetrating foreign body injury limbal laceration involving lens retinal tear vitreous hemorrhage encircling band procedure with scleral buckling development of fibrous membrane behind lens	I I	20/70

Retinal fibrotic scar and preretinal traction explaining poor visual acuity postoperatively
Patient lost to follow up Fundus view immediately after operation

Discussion

In this study we have presented 15 cases of intraocular *trauma* many of which were refractory to traditional methods of treatment The three major categories of trauma presented which eventually necessitated the use of the vitrophage were (1) traumatic secondary membrane formation (cases 1 2 5 8 10 14 15) (2) dislocated or traumatized lens (cases 2 1 6 7 9 10 11 13–15) (3) vitreous hemorrhage and retinal injury (cases 3 4 7 10 11 14 15) As can be seen there is extensive overlap between these three groups This would be expected due to the severe nature of the ocular trauma A case history representative of each group was presented

Groups 1 and 2 responded well to vitrectomy alone (Figs 1 and 2) The vitrophage is very efficient in removing the traumatized lens and secondary membrane Iris adhesions in these two groups were cut without complications Unfortunately the management of group 3 was not as successful in all cases (Fig 4) In case 10 retinal detachment was discovered after vitrectomy Massive preretinal membrane formation and fixed folds made reattachment impossible In cases 14 and 15 the injury that caused retinal detachment was in the fundus periphery thus it was possible to reattach the retina with a combination of vitrectomy intravitreal gas injection and an encircling procedure Although case 11 showed excellent visual improvement after early vitrectomy the retina detached later because of massive preretinal membrane formation The retina was successfully reattached and there have been no subsequent problems Case 7 did not sustain penetrating trauma Although there was a temporal ddisinsertion of the retina cryocoagulation prevented retinal detachment

Cases 4 10 and 11 illustrate that early vitrectomy alone is not always sufficient to preserve useful vision in the presence of retinal injury In cases such as these we feel it is important to diathermize the involved area of the retina immediately following vitrectomy or photocoagulate *the involved area* within 24 hours In case 4 poor postoperative visual acuity may have been averted had the trauma been treated more aggressively

None of the 15 patients had complications during surgery related to the vitrophage Minimal problems occurred postoperatively At the site of laceration in case 12 there were early signs of vascularization and punctate staining of the cornea Any cases of transient glaucoma were successfully treated with acetazolamide and/or topical antiglaucoma agents

Previously other accepted techniques for these aforementioned injuries have had an anterior approach (Chandler Cox et al Shock et al Zagora) which has led to vitreous loss further membrane formation and inadequate exposure

Furthermore in cases of old trauma the lens pupillary and fibrous membranes are resistant to removal and difficult to handle by usual methods

We feel that one of the major advantages to pars plana lensectomy membranectomy and vitrectomy is the small incision In vitrectomy the eye is subjected to minimal manipulation and further trauma In addition we feel that removal of the intraocular debris and involved vitreous helps prevent subsequent membrane formation and hastens visual rehabilitation

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ANTERIOR CHAMBER DEPTH AND PRIMARY ANGLE CLOSURE GLAUCOMA

II A genetic study

BY

P H ALSBIRK

The genetics of primary angle closure glaucoma (a c g) was studied a) through the prevalence in sibs and children of a c g probands and b) through the family distribution of the closely correlated axial anterior chamber depth (ACD) The material emerged from an epidemiologic study in Greenland Eskimos

a) Compared with the general population the observed prevalence of a c g was increased in sibs of a c g probands and the estimated future prevalence was found to be the same in sibs and children. Age influence prevented a proper Mendelian analysis but no simple monogenic inheritance seems probable

b) The biometric study showed a relatively shallow chamber in sibs children nephews nieces and grandchildren of a c g probands Regression analyses revealed a corresponding pattern also in control families of probands with shallow chambers and in general population families A heritability of 70 % was found indicating that about two thirds of the age and sex independent variation in ACD seems to be genetic

Key words genetics – angle closure glaucoma – anterior chamber depth – family prevalence – heritability – multifactorial inheritance

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Familial occurrence of primary angle closure glaucoma (a c g) is well known but the true frequency is obscure because epidemiologic studies of this disease have been lacking The few clinical case series available do demonstrate an increased frequency in sibs of Caucasian a c g probands with prevalence rates ranging from 5–10 % among the sibs examined (Tornquist 1953 Paterson 1961 Perkins 1974) On the other hand Lowe (1964) judging from interviews with 200 Australian White a c g patients reported that only 4/778 siblings (i e 0 5 %) with acute or subacute a c g were known but the sibs registered were not examined None of these studies were able properly to compare the prevalence in sibs with that of the background population As to children and other relatives our knowledge is even less mostly due to the late age of manifestation However numerous studies of single pedigrees which probably represent the exceptions rather than the rule how transmission through two or more generations and irregular autosomal dominance has been the favoured mode of inheritance (e g Westerlund 1947 François 1961) Recently Lowe (1972) advanced the hypothesis of polygenic inheritance with a threshold effect as a more likely model

Until now only a few investigators have considered the *inheritance of the ocular dimensions* in spite of several connections between ocular anatomy and disease Thus a c g is associated with a shallow narrow angled anterior chamber Tornquist (1953) demonstrated a significant shallowness also in sibs and children of probands with acute glaucoma Furthermore he studied the axial anterior chamber depth (ACD) in twins (without a c g) The variation within monozygotic and same sex dizygotic twin pairs differed significantly The data indicate that the main part about 70 % of the variance in dizygotic twins seems to be genetic Twin studies and family correlation studies of the refractive components including ACD made by Sorsby et al (1964 1966) seem to agree with this finding On the other hand the heritability studies by Nakajima et al (1968) from Japan suggested only an insignificant genetic influence on ACD irrespective of whether twins or parent offspring groups were considered Young & Leary (1972) found a significant positive parent offspring correlation (+0 24) of ACD in Alaska Eskimos but they were not inclined to take this and other similar oculometric correlations as indicators of a genetic influence Recently Tomlinson & Leighton (1973) showed in a small sample of sibs and children of a.c.g probands that ACD corneal diameter and height as well as axial length were shorter and lens thickness higher than in a sample of controls Of these parameters ACD showed the relatively largest deviation

Family studies in primary open angle glaucoma on the genetic determination of characters such as the intraocular pressure outflow facility and topical corticosteroid response have valuably elucidated the inheritance of this disease

(e g Armaly 1968 Phelps & Podos 1974) As to a c g much remains to be done In a recent epidemiologic study the remarkable prevalence of a c g in Greenland Eskimos was analysed and related to the general level of the ACD distribution (Alsbirk 1975) Unlike earlier genetic studies of a c g the probands derived from a population survey Based on the same material the present study concerns *a)* the occurrence of a c g in sibs and children as well as *b)* the family distribution of ACD

Material

The 60 a c g probands of this study lived in seven medical districts in East and West Greenland They were independently ascertained by the diagnosis of a c g found in the ophthalmic files or by two population screenings The proband sample has recently been described in terms of age sex ACD and method of detection (Alsbirk 1975) The patients were *Eskimos* in the wide sense previously described (Alsbirk 1974a)

The *relatives of 52 a c g probands* were examined as shown on the left in Tables I–IV (Eight of the 60 probands without relatives were thus excluded)

Out of 206 *sibs* and *children* present at the time of the study *199 (93 %)* were examined Reliable information on a c g was not obtainable for 214 deceased and 107 moved or absent sibs and children Unlike the groups of first degree relatives more distant relatives were not systematically traced in all the districts However in Umanaq *56 second degree relatives* happened to be included in the ACD population survey and their data were thus available also for the present study (Tables III–IV) The *spouses* of a c g probands were examined whenever possible Apart from deceased (19) absent (7) or non Eskimo spouses (2) 24/27 were examined all but one having children who were also measured

The biometric pattern outside a c g families was studied for purposes of comparison The *control families* were obtained through the ACD population survey in Umanaq district (Alsbirk 1974a) which covered 931 persons i e 98 % of town Eskimos over 7 years and village Eskimos over 40 years For the present study two different control groups were examined

1) *Shallow chamber probands* were selected by the following criteria all persons in the ACD survey *aged 40 or more without a c g* but with an average *ACD value below 2 0 mm* Thus 54 probands – 20 ♂ and 34 ♀ – were selected who made up 9 % (20/215) of the males and 16 % (34/213) of the females in the Umanaq ACD survey population over 40 years of age A total of 220 first degree relatives 133 second degree relatives and 32 spouses of these probands was thus examined (One of the probands had no relatives present and was excluded) Again sibs children and spouses had been

systematically traced while only those second degree relatives who belonged to the ACD survey populations were included (Tables I IV)

2) *General population marital pairs* without a c g and without a c g patients among first degree relatives were selected provided that husband and wife were not related (as close as second cousins) A total of 77 pairs with at least one child examined was selected in this way for a separate child on midparent and husband on wife regression study (Table V)

Whenever both eyes of an *individual could be reliably measured the average* ACD value was taken as the genetically most relevant measure The small side difference with deeper left eyes recently described (Alsbirk 1974c) was systematically corrected for in the few per cent of persons in whom only one eye was measured

Methods

The ACD measurement was obtained by optical pachymetry as previously described (Alsbirk 1974c) The *diagnostic criteria* were given in the epidemiologic paper (Alsbirk 1975) A c g had been diagnosed as latent intermittent acute or chronic at detection – a subdivision which will be disregarded here as these clinical subgroups were homogeneous with regard to ACD

Pedigrees of the a c g patients and all families in the ACD survey of Umanaq district were collected Parents full and half sibs spouses and children were recorded and consanguinity specified In the Umanaq pedigrees the information was generally collected through the females Extramarital children were included in child on mother not in child on father regression analyses As a rule the interrogated persons co operated willingly Very often a check was obtained through interviews with relatives

Statistical analyses As the ACD distribution had been shown to depend on age sex and partly on location (Alsbirk 1974a b) the ACD data at hand could not be used directly As earlier described (Alsbirk 1975) a standardized deviation score (DS) was calculated for each person based on the linear regression of ACD on age in three age groups 7–14 15–39 and 40+ of the general population separately for each sex Further the location groups which differed from the others – Angmagssalik males by their ACD variance and Sukkertoppen females by their mean value (Alsbirk 1974b) – were treated separately while the others were pooled The Gaussian distributions obtained in this way had means ($\overline{DS}$) at zero and standard deviations (s_D) at unity (1 0) in subgroups of the general ACD survey population Thus the total group of 1,570 persons showed the values $\overline{DS} = 0\,00$ $s_{DS} = 0\,99$ $s_{\overline{DS}} = 0\,025$

Family distributions of ACD were studied using the age and sex independent DS values Firstly the DS distributions in various groups of relatives were compared with the population parameters Secondly the association between DS values in probands and relatives was gauged by means of regression statistics In the analyses of a c g and shallow chamber families the probands had been selected from the lower tail of the ACD distribution and therefore the regression coefficients (b_y) of straight lines through the origin were found from $b_y = \Sigma xy/\Sigma x$ (x = DS value of the proband y = DS value of the relative) (Snedecor & Cochran 1967) Correspondingly the standard error was estimated from $s_b = (\Sigma y - (\Sigma xy)^2/\Sigma x)/((n\ 1)\ \Sigma x)$ where n = no of correlated pairs

Table I

Primary angle closure glaucoma (a c g) in sibs and children of probands with a c g *(left)* or with shallow chambers without a c g *(right)* Sample sizes and age composition are shown

A c g probands		Proportion of relatives with a c g			Shallow chamber probands		Proportion of relatives with a c g		
no	sex	♂	♀	♂+♀	no	sex	♂	♀	♂+♀
		Sibs					Sibs		
6	♂	0/4	1/7	1/11	10	♂	1/4	1/13	2/1[illegible]
24	♀	1/16	4/22	5/38	16	♀	0/23	0 9	0 3[illegible]
30	♂+♀	1/20	5/29	6/49	26	♂+♀	1/27	1/2[illegible]	[illegible]/49
		Children					Children		
9	♂	0/14	1/16	1/30	19	♂	0/34	0/2[illegible]	0/56
38	♀	0/49	0/64	0/113	31	♀	0 65	0/50	0/115
47	♂+♀	0/63	1/80	1/143	50	♂+♀	0/99	0/7[illegible]	0/171

Age median & range ( )

Probands	60 5	(40–84)	Probands	60	(40–84)
Sibs	51	(33–75)	Sibs	57	(33–84)
Children	29	(8–60)	*Children*	29	(8–56)

Numerators show no of affected persons out of the groups given in denominators

Results

The results are given in Tables I–V with a c g families on the left hand side and shallow chamber families on the right

Prevalence of a c g in sibs and children

Table I shows the number and proportion of a c g patients in these groups In the a c g families only three sibships were found with two affected sibs all of whom were probands According to Weinberg s proband method the sib sample showed a total prevalence of 12 % (6/49) against an overall population value of 3 5 % over 40 years (Alsbirk 1975) The age composition of the sib sample was slightly younger than that of the ACD survey population over 40 years

(mean 55) Nevertheless the prevalence at least in female sibs was significantly higher than the population value 5/29 = 17 % against 39/765 = 5 1 % (χ^2 = 5 8 $P < 0.05$) The families of the shallow chamber probands contained 2/49 affected sibs and did not differ significantly either from a c g families or from population value

Generally the *actual prevalence* of a c g was low or zero in most groups As to the children however only a few had attained even the lower range of onset age characteristic of the a.c g patients *Expected future prevalence* rates in the same groups of relatives were calculated for female relatives according to the DS distributions and the risk of a c g at various levels of DS given in Table II

Table II

ACD as female DS distributions with percentual risk of a c g at various levels of DS Expected prevalence of a c g is given in relatives of probands with a c g and with shallow chambers only

Lower class limits of DS	General population (ACD survey)			A c g probands		Shallow chamber probands		Total No
	A c g patients no	Total no	Risk of a c g	Sibs no	Children no	Sibs no	Children no	
+3 00		1						
+2 50		4						
+2 00		13			1			1
+1.50		26			2	1	2	5
+1 00		77		2	5	–	2	9
+0 50		121		4	7	4	7	22
0 00	1	148	*0 7 %*	6	16	7	12	41
–0.50	1	142	*0 7 %*	4	15	1	14	34
–1 00	6	116	*5.2 %*	6	10	4	13	33
–1 50	13	73	*17.8 %*	4	14	2	10	30
–2 00	9	27	*33.3 %*	1	10	1	7	19
–2 50	6	11	*59.9 %* (–2 50 to <–3 00)	2		2	5	9
–3 00	1	4						
<–3 00	2	2						
Total no	39	765		29	80	22	72	203
Prevalence rate		5 %		9 %	8 %	9 %	11 %	9 %

The risk figures (in per cent) were obtained as for ACD in a previous paper (Alsbirk 1975) by the proportion of a c g patients at various DS levels Thus at each level the *risk percentage out of number of relatives gave the expected number of affected relatives* When summed at all levels the expected prevalence was obtained According to this method 9 % out of all groups of female relatives have probably developed a c g when in the future the age composition of the groups has attained that of the basic a c g material This value is slightly higher than the population value 5 % (χ 4 1 $P < 0\ 05$) No difference between any of the subgroups appeared (a c g versus shallow chamber families or sibs versus children) The analysis could only be made in females as only five male a c g patients were found in the ACD survey

Familial ACD distributions

The family distributions are given as DS parameters in Table III according to *a c g probands* or *shallow chamber probands* with the sexes pooled as no difference was found The same pattern emerged in both types of families The sib $\overline{DS}$ values were equal and the difference between children s groups was not significant Judged by the standard errors ($s_{\overline{D}}$) most groups of relatives differed significantly from zero towards the negative proband mean values On the other hand the spouses showed positive means The spouses of the a c g probands differed significantly from zero ($P < 0\ 01$) and from the other spouse sample ($P < 0\ 05$)

In Table IV the resemblance between relatives and the two types of probands is assessed by regression analyses Furthermore child on midparent analyses are included Thus the difference between the two child on proband values ($P < 0\ 05$) clearly disappeared when both parents were considered Pooled estimates from both proband groups are shown at the bottom

General population families without a c g covering the whole range of ACD values are shown in Table V with sons and daughters pooled as no *difference* was found None of the mean values ($\overline{DS}$) differed significantly from zero Ordinary within sample regression and correlation coefficients were calculated and were found to agree with the values of Table IV Also this child on parent regression (0 39) was significantly higher than in a c g families (0 21) In the same 77 families the resemblance of the siblings in the children s generation was studied The intraclass correlation coefficient (r_I) was found to be 0 1? in 63 pairs of brothers and 0 34 in 69 pairs of sisters – *when disregarding sex* r_I = 0 28 (291 pairs) in agreement with the sib on proband regressions of Table IV

The *marital regression coefficient* was again nearly zero (+0 12 Table V) In order to get a more reliable estimate of this value all marital Eskimo pairs in the Umanaq ACD survey were considered A total of 191 pairs was left when 22 consanguineous pairs had been excluded The overall husband on wife

Table III

ACD as DS parameters in family groups ascertained by a c g probands or shallow chamber probands

Sample of	DS in a c g families				DS in shallow chamber families			
	no	$\overline{DS}$	$s_{\overline{DS}}$	s_{DS}	no	$\overline{DS}$	$s_{\overline{DS}}$	s_{DS}
Probands	52	−1.57	0.10	0.79	53	−1.49	0.07	0.51
Full sibs	49	−0.40	0.14	0.96	49	−0.39	0.13	0.89
Children	149	−0.32	0.08	0.94	171	−0.17	0.07	0.91
Total (1st degree)	192	−0.34	0.07	0.94	290	−0.46	0.06	0.90
Nephews & nieces	28	−0.46	0.22	1.14	75	−0.12	0.11	0.98
Grandchildren	28	−0.33	0.22	1.19	58	−0.05	0.11	0.83
Total (2nd degree)	56	−0.39	0.15	1.15	133	−0.09	0.08	0.92
Spouses	23	0.55	0.19	0.91	32	0.06	0.14	0.79

$\overline{DS}$ mean value of deviation scores (DS) $s_{\overline{DS}}$ standard error of the mean s_{DS} standard deviation

Pooled estimates 1st degree (n = 419) $\overline{DS} \pm s_{\overline{DS}} = -0.40 \pm 0.05$

2nd degree (n = 189) $\overline{DS} \pm s_{\overline{DS}} = -0.18 \pm 0.07$

Table IV

ACD as DS parameters in family groups as in Table III Relative on proband regressions (b_{yx}) with standard errors (s_b) are given

Relationship (y) (x)	A c g families			Shallow chamber families		
	No of yx pairs	b_{yx}	s_b	No of yx pairs	b_{yx}	s_b
Child on midparent	82	0.68	0.14	92	0.44	0.11
Sib on proband	49	0.31	0.08	49	0.79	0.08
Child on proband	143	0.21	0.04	141	0.36	0.05
Total (1st degree)	192	0.23	0.04	290	0.34	0.04
Nephew or niece on proband	28	0.30	0.12	75	0.14	0.08
Grandchild on proband	28	0.23	0.13	58	0.08	0.10
Total (2nd degree)	56	0.27	0.03	133	0.12	0.06
Spouse on proband	23	−0.29	0.11	32	−0.03	0.09

Pooled estimates

Child on midparent (n = 174) $b_{yx} \pm s_b = 0.71 \pm 0.09$

1st degree on proband (n = 412) $b_{yx} \pm s_b = 0.28 \pm 0.03$

2nd degree on proband (n = 189) $b_{yx} \pm s_b = 0.19 \pm 0.05$

regression was $b_{yx} \pm s_b = -0.03 \pm 0.08$ The Figure illustrates the mutually independent DS values of this material against the highly positive correlation between children and midparent values from the material of Table V

Discussion

The genetics of primary angle closure glaucoma (a c g) was studied in this paper from both a qualitative and a quantitative point of view a) through the prevalence of a c g in sibs and children of a c g probands and b) through the family distributions of ACD

Table V
ACD correlation in general population families without a c g

Relationship (y) (x)	No of pairs	b_{yx}	s_b	Correlation coefficient r
Child on midparent	214	0 78	0 09	0.50
Child on father	214	0 45	0 07	0 40
Child on mother	214	0 34	0 07	0.31
Child on parent	428	0 39	0 05	0.35
Husband on wife	77	0 12	0 12	0 12

The first part of the study did reveal a slightly *increased (12 %) sib prevalence of a c g* 3 5 times higher than the population value However for several reasons a proper Mendelian analysis could not be made Firstly the disease had not until recently been generally recognized by the health service in Greenland

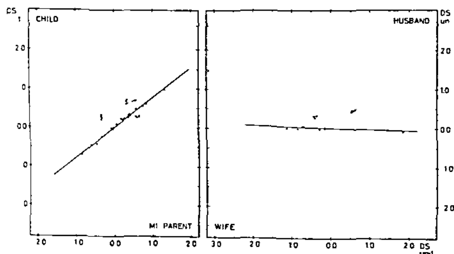


Fig 1
Child on midparent versus *husband on wife* scattergrams of anterior chamber depth (ACD) plotted as the age and sex independent standardized deviation score (DS) A highly significant regression is shown in the left graph (b = 0 78)

Therefore the ocular state of earlier generations could not be included Secondly the great *age influence* on the prevalence is generally a serious problem when attempting to estimate familial prevalence rates Stromgren (1935) presented a method for age correction of a sample of relatives according to the age at onset of a disease The present samples of female sibs (29) and children (80) of a c g probands shrunk to nine persons and one person respectively when the individual 5 year age groups were weighted according to the onset age distribution of 50 female a c g patients Evidently a much larger cross sectional material should be available – or a longitudinal study should be undertaken – in order to obtain the reliable familial prevalence rates which are necessary for statistical comparisons with hypothetical Mendelian ratios

With this background the *expected prevalence rates* based on the ACD measurements were of interest (Table II) Probably a slightly elevated family prevalence of a c g must be expected of equal size in sibs and children As many as 10 % of these should become a c g patients It must be emphasized however that this estimate is bound to the age pattern of the group of probands Increased life expectancy of the younger generation might increase the prevalence However as a whole the figures presented fell far short of the 50 % prevalence to be expected according to a hypothesis of regular autosomal dominant inheritance Fraser Roberts (1961) stated – the diseases and mortality of post reproductive life show little if any evidence of simple Mendelian ratios and this holds true of a c g

The concept of *polygenic* or *multifactorial inheritance* is largely based on studies of the inheritance of quantitative characters (cf below) Recently however an important further development has taken place through the heritability of liability" model which seems to fit the family pattern of qualitative conditions found in many common disorders much better than monomeric Mendelian models (Falconer 1965 1967 Edwards 1969 Smith 1971) The model was reviewed in an ophthalmological context by Grutzner et al (1970)

The heritability of liability model makes use of the ratio of prevalence in relatives to prevalence in population Especially in common disorders with population prevalence exceeding 1 % as in the disease under study a considerable degree of genetic determination may be present even if the prevalence excess in relatives is small (Falconer 1965 Edwards 1969) An estimate of the heritability (h^2) of a c g in females by means of Falconer s method was made (based on 5/29 actually affected female sibs (*Table I*) against 39/765 affected females of the general population (Table II)) However the standard error largely invalidated the estimate thus obtained (h = 0 7 ± 0 3) In conclusion genetic determination was only suggested when the a c g occurrence was analysed by the qualitative methods

The previous epidemiologic study revealed a close association between level of ACD and prevalence of a c g The sex difference and ethnic variation in

prevalence of a c g seemed to reflect the ACD distributions (Alsbirk 1975) Therefore a *quantitative genetic study* of the familial ACD distribution was a natural second part of this study As Tables III IV and V show a fairly clear pattern emerged

Nearly 200 *marital pairs* were examined Ideas of assortative mating and/or environmental influence common to husband and wife received no support by this study as the regression was almost zero For the time being the amazingly deep chambers of the few spouses of a c g probands (Tables III IV) must be taken as the effect of sampling variation

On the baseline of non assortative mating the positive regression coefficients of various relatives on a c g probands revealed a considerable genetic influence Thus the finding of Tornquist (1953) were essentially confirmed in a larger material of a c g families from a new population Also Eskimo sibs and children deviated significantly towards the low negative DS values in the a c g probands (Table III) Furthermore nephews nieces and grandchildren showed a similar trend However regression methods and a control sample of shallow chamber families were used in the present study for the first time As *a main result* it was found that the *shallow chamber in relatives of a c g probands simply reflects a general pattern which is found just as markedly in other families* whether these are selected from the lower tail or from the whole range of the ACD distribution

The genetic interpretation of these results seems to be fairly clear *The heritability* $h = V_A/V_P$ indicates the amount of additive genetic variance (V_A) as a proportion of the total phenotypic variance (V_P) in a trait and ranges thus from zero to unity (e g Falconer 1965) The family correlation coefficients estimate $0\ 5 \times h$ in first degree and $0\ 25 \times h$ in second degree relationships (the numerical factor indicates the proportion of genes in common) By means of the age and sex independent deviation score large groups could be pooled together The regressions (b_{yx} of the Tables) could generally be taken as correlation coefficient estimates because the DS values had been expressed in terms of standard deviation from regression According to these principles the family patterns of Tables IV and V gave a fairly consistent heritability estimate of ACD The pooled a c g and shallow chamber families gave $h^2 = 0\ 57 \pm 0\ 06$ (1st degree) and $h = 0\ 75 \pm 0\ 21$ (2nd degree) According to theory the child on midparent regressions directly estimate h and values close to 0 7 were obtained in all sub groups (In this particular relation the correlation $r = 0\ 50$ (Table V) estimates $h/\sqrt{2}$ i e $h = 0\ 7$ as before) Generally sib on proband regressions agreed with child on proband values The former would have been larger if a common family environment had markedly influenced the anterior chamber or if a significant dominance component was a part of the genetic determination

However the standard errors of the subgroups were all rather large and a number of details may still be obscure Thus males and females were pooled in most comparisons as no sex difference had been found However when the sex difference in a c g prevalence is remembered a variation in male and female relatives of male and female probands might possibly be expected This and other related problems as well as certain anthropological aspects are under study

In conclusion the present study showed that a slightly elevated prevalence of primary angle closure glaucoma (a c.g) is found in sibs and can be similarly expected in children of a c g probands This pattern is in agreement with the concept of multifactorial inheritance but only vague indications could be obtained from the qualitative data On the other hand an underlying metric trait ACD displayed a uniform distribution in families with a c g as well as in other families and this pattern indicates a fairly high degree of genetic *determination A heritability estimate of about 0 7 was obtained i e about two* thirds of the age and sex independent ACD variation seems to be genetically determined

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CONJUNCTIVAL SENSITIVITY IN PATHOLOGICAL CASES

With Simultaneous Measurement of Corneal and Lid Margin Sensitivity

BY

M S NORN

A total of 209 pathological eyes each had 17 localities tested for sensitivity (cornea caruncle upper and lower lid margins (centrally medially and laterally) and corresponding localities on the palpebral conjunctiva and upper and lower halves of the bulbar conjunctiva)

Reduced *conjunctival* sensitivity is seen in pemphigoid (excluding the lid margin) in contact lens wearers at sites of nerves transected during operation and in rare cases of infectious conjunctivitis

Isolated *corneal* hypaesthesia is seen in bacterial or fungal keratitis In herpes the hypaesthesia extends over the bulbar conjunctiva in zoster over wider areas (including the lid margin)

The sensitivity is normal in keratoconjunctivitis sicca and chronic conjunctivitis

In neurological diseases the hyposensitivity could include the cornea conjunctiva and lid margin

The conclusion is drawn that a study of the conjunctivo corneal sensitivity can give differential diagnostic information provided the normal sensitivity range is known This has been set out in a Table in 10 year age groups

Key words conjunctiva – cornea – sensitivity aesthesiometer – pathological cases

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Many investigations have been published concerning the corneal sensitivity whereas only a few concentrate on the conjunctival

In a previous paper (Norn 1973) I reported the results of an investigation into the conjunctival sensitivity of 102 normal subjects The sensitivity declines with increasing age It is highest on the cornea and lowest on the conjunctiva The lid margin sensitivity is intermediate between these two The various conjunctival regions (palpebral bulbar medial lateral upper lower) are equal in sensitivity as are also those of the lid margins (upper lower medial lateral central)

The object of the present investigation has been to establish a well defined normal range and to study a pathological material on this basis for the purpose of finding out which clinically relevant data are obtainable by testing the conjunctival sensitivity possibly supplemented by testing the corneal sensitivity also

Method

The anaesthesiometer employed is that constructed by Cochet & Bonnet with a nylon thread 0 12 mm in diameter (marketed by the firm Luneau & Coffignon Paris)

The test is first carried out with maximum thread length (60 mm) If the patient does not feel the touch the thread is shortened by 5 mm and so on until the patient is just aware of the touch (minimum perceptibility)

The 17 sites tested are a central point on the cornea nine conjunctival localities (three points located superiorly on the bulbar conjunctiva (medially centrally and laterally) three corresponding points located inferiorly on the bulbar conjunctiva and three corresponding points on the lower palpebral conjunctiva) a further six points on the lid margin (medially centrally and laterally on the upper and the lower lid respectively) and a central point on the caruncle Where abnormal conditions are noticed the site is subjected to detailed examination to disclose the extent of the altered sensitivity

The method is identical with that employed for the previous study of a normal material (Norn 1973 1974)

Normal range

Table I shows the mean values of 102 normal subjects in different age groups (Norn 1973) The sensitivity is expressed in the simplest possible manner (millimeter nylon thread)

Table I

Normal values and normal ranges in parentheses for conjunctival and corneal sensitivities at different ages The figures indicate nylon thread length (in mm) using Cochet & Bonnet s aesthesiometer thread diameter 0 12 mm

Ages	< 40	≧ 40	≧ 50	≧ 60	≧ 70
Cornea	60 (45–> 60)	60 (45–> 60)	55 (45–> 60)	48 (45–> 60)	44 (45–> 60)
Conjunctiva (9 areas)	22 (10–35)	18 (10–30)	17 (10–30)	14 (10–30)	15 (10–30)
Caruncle	28 (20–40)	24 (15–35)	23 (15–35)	18 (10–30)	20 (10–30)
Lid margin (7 areas)	32 (25–45)	27 (20–40)	24 (15–35)	20 (10–35)	21 (10–35)

The normal range shown in parentheses is measured to the nearest 5 mm nylon thread length Cornea and conjunctiva include 96 % caruncle 95 % and lid margin 93 % of the normal material

Result

Hyposensitivity

In this selected pathological material of 209 eyes the following reduced sensitivities were noticed cornea (74 cases) bulbar conjunctiva (45) and inferior palpebral conjunctiva (20) Hyposensitivity of caruncle and lid margin was less frequent (Table II)

Corneal lesions

The sensitivity of the cornea was reduced in most cases of *dendritic keratitis* either diffusely or limited to a fairly small area covering the dendriform pattern In 15 cases the cornea alone was hyposensitive In four cases (Table II) the

Table II

Hyposensitivity of conjunctiva and cornea tested on 209 pathological eyes The figures indicate the numbers of eyes with reduced sensitivity (Normal range Table I)

	Cornea	Bulbar conj	Palpebr conj	Caruncle	Lid margin	Total no hyposensit	Total no eyes
Dendritic keratitis	21	4	2	0	2	21	24
Zoster ophthalmicus	10	6	2	2	4	13	15
Corneal graft	7	1*	0	0	0	7	9
Pemphigoid (B M M P)	3	8	10	4	0	11	15
Infect conjunctivitis	2	2	2	0	0	5	16
Contact lens	0	2	0	0	0	2	20
Cataract extraction	6	9	0	0	0	9	12
Neurol diseases	7	5	3	1	2	8	31
Other diseases	18	8	1	1	2	25	67
Total	74	45	20	8	10	101	209

After dendritic keratitis

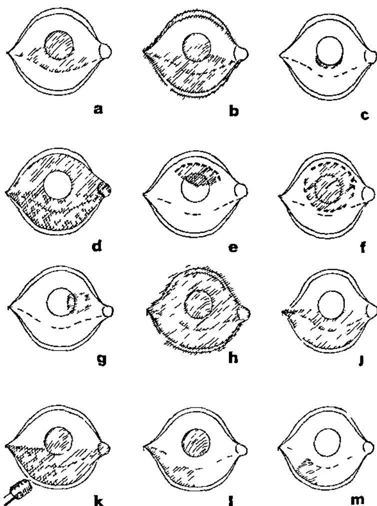


Fig 1
Hyposensitivity of conjunctiva cornea and lid margin Typical clinical pictures Hyposensitive areas hatched and markedly hyposensitive double hatched *a* dendritic keratitis *b* zoster ophthalmicus *c* contact lens wearer *d* pemphigoid (BMMP) *e* cataract extraction *f* cerclage operation a m. Arruga *g* strabismus operation *h* trigeminal block *j* lesion of infraorbital nerve *k m* cocaine 10 % 0 30 and 60 min after application

hyposensitive region extended downwards from the cornea to the bulbar conjunctiva (Fig 1 a) In two cases the corneal hyposensitivity was attended by hyposensitivity of the tarsus and lid margin respectively In the remaining three cases the sensitivity was normal throughout

Zoster ophthalmicus The hyposensitivity was more extensive than in the cases of dendritic keratitis (Fig 1 b) In four eyes only isolated corneal hypo sensitivity was found while in six the hyposensitivity extended from the cornea beyond the bulbar conjunctiva and in some cases included the caruncle and lid margin Two eyes showed isolated hyposensitivity of the tarsus and lid margin and one of the lid margin and caruncle

Other corneal lesions were attended by hyposensitivity of the cornea alone (marginal keratitis corneal opacity corrosion pterygium and graft)

Contact lens wearers

A series of 20 eyes was tested of which four could not tolerate hard corneal contact lenses even though they had normal sensitivity

Hyposensitivity was noticed in two cases only and in both it was localized within a crescent area just below the cornea corresponding to the low riding of the hard corneal lens (Fig 1 c) The corneal sensitivity was normal in all cases

Conjunctival disorders

Benign mucous membrane pemphigoid was in many cases attended by extensive hyposensitivity The reduced sensitivity was not limited to symblepharon bands or plagues but comprised the entire fornix and tarsus from where it even extended to the bulbar conjunctiva and the caruncle

In rare cases the corneal sensitivity was also reduced (with concurrent opacity or conjunctival invasion)

In some cases the conjunctival sensitivity was very greatly reduced almost to the extent of anaesthesia The lid margin sensitivity on the other hand was always normal (Fig 1 d)

In *infectious conjunctivitis* the sensitivity was generally normal We did however in a small number of cases find hyposensitivity of the bulbar or the palpebral conjunctiva or mild corneal hyposensitivity The lid margin sensitivity was normal

The sensitivity was normal in the cases of *simple* chronic conjunctivitis and keratoconjunctivitis *sicca*

Chemosis was accompanied by hyposensitivity over the oedematous region Some cases of ectropion showed hyposensitivity of the ectropionised palpebral conjunctiva

Operations

After *cataract* extraction with limbus based conjunctival flap a significantly reduced sensitivity of the conjunctival flap was noticed in nine out of twelve cases tested within the first postoperative week The hyposensitivity was limited

to the flap itself and to a possibly exposed sclera extending to the upper part of the cornea at the site of the transected nerves The conjunctival sensitivity peripheral to the flap was normal (Fig 1 e)

Operation for *retinal detachment* was in some instances followed by hyposensitivity of the cornea and bulbar conjunctiva all the way round the cornea after the cerclage operation (Fig 1 f) Reduced conjunctival sensitivity was found over the area just above a Custodis silicone implant

After operation for *glaucoma* some patients had persistent hyposensitivity on Elliot s filtration bleb and corneal hyposensitivity was found in cases of corneal dystrophy with bullae

Operation for *strabismus* was sometimes followed by reduced sensitivity at the site of operation and the adjacent part of the cornea (Fig 1 g)

Neurological cases

Some patients with trigeminal nerve block showed anaesthesia of the whole cornea bulbar and palpebral conjunctivae and lid margin (Fig 1 h)

In a case of fracture of the lower orbital border with associated lesion of the infra orbital nerve the corneal sensitivity was normal while there was hypaesthesia of the palpebral conjunctiva and the lower half of the bulbar conjunctiva (Fig 1 j)

Experimental studies

The effect of swabbing 10 % cocaine laterally onto the inferior tarsus for 2 or 3 min was studied at different intervals for 1 hour (Fig 1 k m)

A state of hypaesthesia to anaesthesia lasted for 2 to 10 min for the cornea, the conjunctiva and the caruncle while the sensitivity of the lid margins remained normal

After 20 min the sensitivity of the upper half of the bulbar conjunctiva had returned to normal The sensitivity of the remaining part of the bulbar conjunctiva and the nasal half of the palpebral conjunctiva returned after 30 min After 60 min the corneal sensitivity was normal Only the area at the site of application located infero laterally on the inferior tarsus remained hypaesthetic

Instillation of Novesin® (oxibuprocaini chloridum NFN) into the inferior fornix likewise caused anaesthesia of the cornea and conjunctiva especially inferiorly The lid margin remained unaffected The anaesthesia lasted up to 20 min

Discussion

Hypersensitivity of the conjunctiva was demonstrated in only a few of the present pathological cases hardly significantly more often than in a normal material Hypersensitivity of the lid margin may presumably be a factor to be considered in the fitting of contact lenses Dixon (1964) and Lowther (1968) showed that the sensitivity may decrease during the fitting period

Hyposensitivity was noticed in many cases not only of the cornea, but also of the other regions More reliable clinical data are obtainable by testing the cornea the conjunctiva and the lid margin for sensitivity than by testing the cornea alone

In corneal hypaesthesia of uncertain origin the conjunctival sensitivity test can give differential diagnostic suggestions For example isolated hypaesthesia of the cornea indicates sequelae of bacterial or fungal keratitis corneal hypaesthesia including a minor part of conjunctiva bulbi suggests herpetic keratitis involvement of a larger conjunctival area even as far as the lid margin suggests zoster In neurological diseases the hyposensitivity could include cornea conjunctiva and lid margin

Chronic conjunctival complaints might occasionally be suspected of being due to increased sensitivity This is not so however These patients have normal conjunctival sensitivity

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BACTERIAL FLORA IN RELATION TO CATARACT EXTRACTION

I Material Methods and Preoperative Flora

BY

J A FAHMY S MØLLER and M WEIS BENTZON

The conjunctival flora of 499 patients was studied the day before cataract extraction no antibiotics or chemotherapeutical agents had been used before admission *Staphylococcus albus* was by far the most common micro organism (95 4 %) followed by corynebacteria (44 %) *Staphylococcus aureus* (14 9 %) gram negative bacilli (7 8 %) and pneumo streptococci (4 4 %) Corynebacteria was isolated more frequently in the presence of *S albus* while *S aureus* and gram negative bacilli were found more frequently in the absence of *S albus* No relationship could be demonstrated between the occurrence of pneumo streptococci and *S albus*
The flora of the nose and skin of the face were studied and compared with the conjunctival flora and a similarity could be observed Furthermore strains of *S aureus* isolated at the same time from the two or three regions in most cases showed the same bacterio phage type complex
The conjunctival flora was further correlated with sex age season and number of polymorphonuclear neutrophils recovered from the conjunctival fluid The incidence of corynebacteria and gram negative bacilli was found to be higher in males while corynebacteria was the only organism to show seasonal variation i e was isolated more frequently in the second and third quarters of the year No correlation could be found between age or number of neutrophils

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Key words conjunctiva – bacteria – flora – cataract extraction – neutrophils

The following series of investigations was undertaken to determine the changes which may occur in the conjunctival flora of eyes undergoing cataract extraction

The bacterial flora was studied on admission (present paper) in the operating theatre (Fahmy et al 1975c) during the stay and finally on the discharge from the hospital (Fahmy et al 1975d)

Further cultures were obtained from the air of wards and operating theatre hands and gloves of surgeons and assistant nurses nasal flora of the staff and eye medications

The present paper which is the first in a series deals with the conjunctival flora as seen on admission its relationship to sex age time of year as well as to the flora of the nose and skin of the face Finally it correlates the incidence of the various bacteria harbouring on the conjunctiva with the number of polymorphonuclear neutrophils found in the conjunctival fluid

Material and Methods

The material comprises 499 patients admitted to the Department of Ophthalmology Kommunehospitalet Copenhagen, during the period 17 8 19 2 to 16 8 1973 for uncomplicated senile (or presenile) cataract extraction, presenting no sign of ocular infection

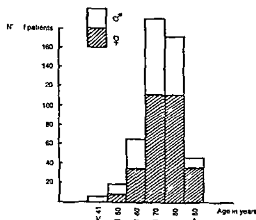


Fig 1
Age and sex distribution of 499 patients undergoing cataract surgery

The material represents 89 3 % of the total number of cases (559 patients) operated upon during this period Sixty patients were not examined as they were admitted when one of the authors (J.A F) was either ill or on holiday

Twenty seven patients were readmitted for operation on the other eye, out of these 21 were reexamined This made a total of 520 examined eyes

However for the sake of simplicity only data collected from the first admission were included Fig 1 shows that the 499 patients comprised 303 (60 7 %) females and 196 males (39 3 %) 408 patients (81 8 %) were over 60 years

Table I

Scheme of sampling and number of specimens obtained from 499 patients undergoing cataract surgery

Code	Origin	Time of sampling	Method	Specimens	
				No	%
A	Conjunctiva	On admission	Dry cotton swab	498	99 8
B	Nose	On admission	Wet cotton swab	498	99 8
C	Skin of face	On admission	Wet cotton swab	497	99 6
D	Air (ward)	On admission	Settling plate	471	94 4
E	Conjunctiva	Start of surgery	Dry cotton swab	496	99 4
F	Air (theatre)	During surgery	Settling plate	488	97 8
G	Surgeon ungloved finger samples	End of surgery	Agar plate	494	99 0
H	Assistant nurse *ungloved finger* samples	End of surgery	Agar plate	493	98 8
I	Wound site	End of surgery	Dry cotton swab	495	99.2
J	Conjunctiva	4th postop day	Dry cotton swab	481	96 4
K	Nose	4th postop day	Wet cotton swab	484	9 0
L	Air (ward)	4th postop day	Settling plate	463	92 8
M	Conjunctiva	7th postop day	Dry cotton swab	483	96 8
Gh*	Surgeon gloved** finger samples	End of surgery	Agar plate	83	98 8
Hh*	Assistant nurse gloved** finger samples	End of surgery	Agar plate	86	98.9

* Examined from 7 5 1973–16 8 1973 total number of patients 84 and 87 respectively

** If any were used

Clinical methods The eyes were examined by the slit lamp Before any bacteriological culture was taken, a cytologic sample from the conjunctival fluid of each patient was obtained and prepared in the same manner as described by Norn (1960)

Sampling methods Table I shows that 13 standard samples were obtained from each patient (at the end of investigation 15) five from the conjunctiva (samples A E I J M) two from the nose (samples B K) and one from the skin of the face (sample C) Three samples were taken from the air surrounding the patients two from the wards (samples D and L) and one from the operating theatre (sample F) Furthermore samples were taken from the ungloved fingers of the operating surgeon (sample G) and assistant nurse (sample H) at the end of the operation In the last 3 months of the investigation (7.5 1973 to 16 8 1973) further samples were taken from the gloves of the surgeon (sample Gh) and assistant nurse (sample Hh) after surgery

Table I shows the number of specimens obtained from the 499 patients it can be seen that the rate of examined specimens varied from 97 8 % to 99 8 %

The methods of taking samples in the present study were as follows Immediately after *admission* and after the instillation of 0 2 % oxybuprocaine (benoximate hydro chloride) anaesthetic eye drops (without preservatives) a culture was obtained from the lower fornical and tarsal conjunctiva (sample A) with a dry cotton wool swab (Fahmy et al 1974 1975a) Cultures were then taken from one of the nostrils (sample B) and from the skin of the face (sample C) with a wet (0 9 % NaCl) cotton wool swab Shortly after a 13 5 cm petri dish containing 5 % blood agar (sample D) was placed at the bedside of each patient and exposed to the air of the ward for 1 hour

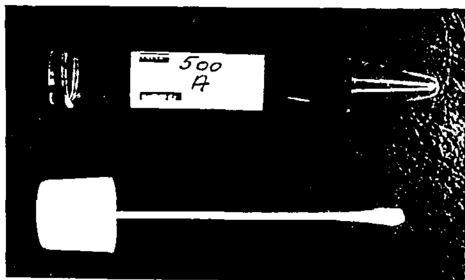


Fig
A cotton wool swab with a container

Bacteriological methods The samples were usually sent immediately (in a few instances within 1–2 hours) to the laboratory for examination The cultures were inoculated in 5 % horse blood agar as well as in serum bouillon and incubated for 48 hours (3, C) Bacteria were identified in the same way as described by Cowan & Steel (1965) However in the present study no attempt was made to identify *Haemophilus influenzae* and anaerobic bacteria as it has been found (Fahmy et al 1974 1975a) that these bacteria are rarely present in normal eyes

Bacteriophage typing of *Staphylococcus aureus* was performed at Statens Serum institut Copenhagen (Department of Hospital Infections) by the methods described by Blair & Williams (1961)

Table II
Incidence of microorganisms isolated from 499 patients prior to cataract surgery

Microorganisms	Incidence in percent					
	Conjunctiva		Nose		Skin of face	
Staphylococcus albus		95 4		87 4		98
Corynebacteria		44 0		65 7		38.5
Staphylococcus aureus		14 8		22 6		9 4
Streptococcus						
haemolyticus	1 8					
non haemolyticus	1 2	4 0	0 2	0 2	0 2	0 8
faecalis	1 0				0 6	
Diplococcus pneumonia		0 4				0 2
Gram negative bacilli						
Escherichia coli	0 8		0 6			
Bacterium anitratum	1 4		1 6		3 6	
Enterobacter cloacae	0 8		0 4		0 2	
Proteus morgani	1 6		0 2		0 4	
Proteus vulgaris	0 4		1 2		0 2	
Proteus mirabilis	1 8	7 8	1 6	6 8	0 6	5 6
Proteus rettgeri	0 2		0 2			
Klebsiella oxytoca	0 6		0 8		0 6	
Klebsiella rhinoscleromatis	0 2		0 2			
Non identified						
gram positive	2 4	2 8	3 2	3 4	4 9	5 0
gram negative	0 4		0 2		0 2	
No growth		none		none		none
Not examined		0 2		0 2		0 4

Statistical methods The results were analysed at the Department of Bio statistics Statens Seruminstitut Copenhagen. In comparing the incidences of the microorganisms on the conjunctiva nose and skin of the face, pairwise comparisons were carried out using the McNemar test In the other comparisons the Chi square test was used In cases where the expected frequencies were below five two or more of the groups were either pooled together or the Fischer exact test was used instead of the Chi square test

Results

Conjunctival preoperative flora

Tables II III and IV show that out of the 499 patients 209 (41 9 %) had one kind of bacteria 235 (47 1 %) two kinds while 54 (10 8 %) had three or more kinds None of the conjunctivas was found to be sterile and in one case (0 2 %) the conjunctiva was not examined A total of 476 patients (95 4 %) had *Staphy*

Table III

Combinations of microorganisms isolated from 499 patients prior to cataract surgery

Microorganisms	Incidence in per cent		
	Conjunctiva	Nose	Skin of face
Sterile	none	none	none
S albus alone	38.5	[illegible]	[illegible]
Corynebacteria alone	0.2	6 4	0.2
S aureus alone	2 2	1 6	
S albus and corynebacteria	33 5	44 4	30 9
S albus and *S aureus*	6 0	9 2	5 0
S aureus and corynebacteria	0 4	2 0	0 4
S aureus and corynebacteria and *S albus*	4 0	8 4	3 3
Other bacteria in various combinations with above	14 0	10 0	11 2
Other bacteria alone	1 0	0 4	0 4
Not examined	0 2	0 2	0 4
Total	100 0	100 0	100 0
One microorganism	41 9	[illegible]	48.3
Two microorganisms	47 1	61 3	43
Three or more microorganisms	10 8	12	[illegible]

Streptococci pneumococci gram negative bacilli and unidentified bacteria

Table IV
Conjunctival preoperative flora of 499 patients prior to cataract surgery

Other microorganisms	*Staphylococcus albus* – corynebacteria – *Staphylococcus aureus*								Not examined	Total
	S albus / *Corynebacteria* / *S aureus*									
	– / – / –	– / – / +	– / + / –	– / + / +	+ / – / –	+ / – / +	+ / + / –	+ / + / +		
None	0	11	1	2	192	30	167	20		423
Streptococcus haemolyticus		2			1	1	3	2		9
Streptococcus non haemolyticus					1	1	4			6
Streptococcus fæcalis					2	2	1			5
Diplococcus pneumoniae					1	1				2
Escherichia coli	1				1		2			4
Enterobacter cloacae	1				3					4
Bacterium anitratum					5		2			7
Proteus morgani	1				4		3			8
Proteus vulgaris					2					2
Pr teus mirabilis	2				6		1			9
Proteus rettgeri					1					1
Klebsiella oxytoca			1				2			3
Klel siella rhinoscler m itis							1			1
Non i lentified gram positive					6		4	2		12
gram negative					1		1			2
N t n n l										1

lococcus albus (coagulase negative) 220 (44 %) corynebacteria 74 (14 8 %) *Staphylococcus aureus* (coagulase positive) 39 (7 8 %) gram negative bacilli 20 (4 %) streptococci 2 (0 4 %) *Diplococcus pneumoniae* while 14 (2 8 %) showed non identified bacteria

Combinations of the different bacteria (Tables III and IV) *S albus* occurred alone in 38 5 % of the cases in combination with corynebacteria in 33 5 % and with *S aureus* in 6 %. All three occurred together in 4 % of the cases Various other combinations between these three bacteria and the other groups (strepto cocci pneumococci gram negative bacilli and non identified bacteria) occurred in 14 % of the cases

Interrelationship of the microorganisms The material was divided according to the occurrence of *S albus* into two groups 476 patients with *S albus* and 22 without (Table IV) *S aureus* was found in 59 cases (12 4 %) in the former group and in 15 (68 2 %) cases in the latter By means of χ test the difference was found highly significant ($P < 0\ 0001$) The relationship of the other groups of bacteria to *S albus* was examined in the same manner corynebacteria occurred significantly more frequently in the presence of *S albus* ($P = 0\ 011$) while gram negative bacilli were found more frequently in its absence ($P = 0\ 0007$) No relationship between the occurrence of streptococci and *S albus* could be seen

Concerning the relationship between the other bacteria Streptococci were recovered more frequently when *S aureus* was present ($P = 0\ 0006$) whereas gram negative bacilli were isolated more frequently when *S aureus* was absent ($P = 0\ 0028$) No significant relationship to corynebacteria could be found

Correlation with sex age and time of year

The material of 499 patients was first divided according to *sex* 196 males and 303 females The incidence of the various groups of bacteria was examined in each sex group and the differences tested by χ test Corynebacteria ($P = 0\ 031$) and gram negative bacilli ($P = 0\ 0086$) were isolated more frequently from males

For the *age* factor the material was divided into six age groups viz ≤ 40 41–50 51–60 61–70 71–80 and > 80 The incidence of the various groups of bacteria was examined and tested (χ test) and no significant difference could be found

For the *seasonal variations* 149 patients were admitted in the first quarter of the year 92 in the second 106 in the third and 152 in the fourth The incidence of the isolated bacteria in the four quarters were compared and tested (χ test) Corynebacteria was the only organism to show a variation i e it was recovered more frequently ($P = 0\ 02\prime$) in the second and third quarters of the year

Relationship between the bacterial flora of conjunctiva nose and skin of the face

Tables II and III show in addition the types and frequency of bacteria cultured from the nose and face skin It is seen that the flora were largely similar to that of the conjunctiva *S albus* was by far the most common followed by coryne bacteria *S aureus* and gram negative bacilli

However the *incidence* of the different microorganisms isolated from the above mentioned locations showed significant variations (Table V) *S albus* (also called *S epidermis*) was found significantly more often on the skin of the face than on the conjunctiva ($P = 0\ 0081$) and nose ($P < 0\ 0001$) *S aureus* and corynebacteria were isolated most frequently from the nose ($P < 0\ 0001$) and streptococci from the conjunctiva ($P = 0\ 0002$) No significant difference was observed between the occurrence of gram negative bacilli and unidentified bacteria on the three regions

As to the relationship of the different bacteria isolated from the conjunctiva and nose *S aureus* was found to occur significantly more often on one site if it was present on the other (χ^2 test $P < 0\ 0001$) The same was true for coryne bacteria ($P < 0\ 0001$) and gram negative bacilli ($P < 0\ 0001$) No correlation could be found between the occurrence of *S albus* and *streptococci*

This close relationship could also be demonstrated in the occurrence of *S aureus* ($P < 0\ 0001$) and gram negative bacilli ($P = 0\ 0002$) on the conjunctiva and skin of the face

Bacteriophage typing of S aureus

In order to study the epidemiological relationship of bacteria harbouring on the conjunctiva to those living on its surroundings and to trace any eye infection which may occur per or postoperatively nearly *all the strains* of *S aureus* isolated from the patients in the present series of investigations were subjected to bacteriophage typing

As seen from Table VI out of 74 strains recovered from the conjunctiva 58 (78 4 %) were typeable whereas 16 (21 6 %) were not Eighteen strains (24 3 %) belonged to group I 10 (13 5 %) to group II 21 (28 4 %) *to group III* and 9 (12 2 %) to the mixed groups The distribution of the strains of *S aureus* isolated from the nose and skin of the face among the mentioned groups was similar to that of the conjunctiva i e out of 113 strains isolated from the nose 86 were typable 21 were not while 6 strains were not phage examined Thirty one (29 %) strains belonged to group I 13 (12 1 %) to group II 28 (26 2 %) to group III and 14 (13 1 %) to the mixed groups Out of 47 strains recovered from the skin of the face 15 (13 9 %) belonged to group I 8 (17 1 %) to group II 15

Table V

Correlation between the incidence of microorganisms isolated from the conjunctiva nose and skin of face of 499 patients prior to cataract surgery

Microorganisms	Conjunctiva (A) Nose (B) *Skin of face (C)*	− − −	− − +	− + −	− + +	+ − −	+ − +	+ + −	+ + +	Not examined	McNemar's test Level of significance A ~ B	A ~ C	B ~ C
S albus		1	5	0	16	2	54	5	414	2	*P* 0.0001	*P* = 0.0091	*P* 0.0001
Corynebacteria		91	28	97	71	44	7	83	56	2	*P* 0.0001	N S**	*P* 0.0001
S aureus		3[illegible]0	3	47	14	7	5	27	95	2	*P* 0.0001	*P* = 0.0007	*P* 0.0001
Streptococci		472	2	1	0	19	3	0	0	2	*P* 0.0001	*P* = 0.0002	N S**
Gram negative bacilli		431	7	11	9	21	4	6	8	2	N S**	N S**	N S**

* Including *Diplococcus pneumoniae* ** Not significant

Correlation between bacteria and conjunctival neutrophils

Table VII correlates the incidence of bacteria with the number of polymorphonuclear neutrophils (PN) obtained from the conjunctival fluid The material was divided into four groups according to the number of PN 179 patients had no PN 250 from 1–99 31 from 100–999 while 12 patients had $\geq$ 1000 PN Thus 43 patients (9 %) had neutrophilia ($\geq$ 100 PN per pipette specimen (Norn 1960)) The specimens of 27 patients were not included either because they were not examined or had shown more lymphocytes than PN

Table VII shows the incidence of microorganisms in each group By means of χ test no correlation could be found as to the kinds of bacteria and number of PN or between the number of colonies of *S aureus* gram negative bacilli and pneumostreptococci and the number of PN

Discussion

In the belief that an accurate knowledge of the natural history of the normal ocular flora is one of the most effective methods for preventing postoperative infections numerous studies have been performed to document the nature of the bacterial flora of eyes about to undergo surgery Table VIII shows some of the results concerning the incidence of microorganisms cultured from the conjunctiva prior to cataract extraction

A common trend can be seen in the preponderance of *S albus* and coryne bacteria in most of the studies (Table VIII) a fact which is supported by this and other investigations on the normal ocular flora (Axenfeld 1907 Pillat 1931 McKee 1924 Lucic 1927 Khorazo & Thompson 1935 Rodin 1945 Barfoed 1953 Smith 1954 Cason & Winkler 1954 Chang 1957 Orfila & Courden 1961 de Ocampo et al 1965 Glawogger 1969 Allansmith et al 1969 Fahmy et al 1974 1975a)

The incidence of *S aureus* seems to be of particular interest as *S aureus* is generally considered to be the major offender in postoperative infections in ophthalmology (Dunnington & Locatcher Khorazo 1945 Locatcher Khorazo & Gutierrez 1956 1960 1967 1972 Burns 1959 Leopold & Apt 1960 Allen & Mangiaracine 1964 McMeel 1965 McMeel & Wapner 1965 Lincoff et al 1965 Allansmith et al 1969) *According to the present study S aureus* occurs in about 15 % of the cases in agreement with some other authors as seen in Table VIII The incidence of 42 % found by Locatcher Khorazo & Seegal (1972) seems to differ significantly from the incidence of the present investigation a fact

Table VIII

Incidence of microorganisms in patients awaiting cataract extraction as found by the different authors

Author	Number of cases	Incidence in percent					
		S albus	Coryne bacteria	*S aureus*	Streptococci§	Gram neg	Negative cultures
Lindner (1914)	500	97 0 *	*	*	46 0	3 2	10
Kraupa (1914)	635	40 0 *	70 0	*	23 0	5 0	33
Stanka (1924)	412	67 8 *	8 2	*	26 4	*	15 7
Edmund (1927)	86	79 9	24 4	69	10 3	2 3	0
v Pelláthy (1939)	1973	65 3	51 1	9 9	17 9	19 2	*
Cooper (1935)	92	84 8	7 0	12 0	1 1	3 2	6 5
Cason & Winkler (1954)	1604	67	33	7 0	4 0	5	23
Doden (1957)	915	75 7	76 4	6 1	2 3	0 8	8 7
Locatcher Khorazo & Seegal (1972)	1641	68 5	28 7	42 0	2 8	3 2	0
Present cases	499	95 4	44 0	14 8	4 4	7 3	0

* Not stated

* A differentiation was not made between *S albus* and *S aureus*

§ *Including pneumococci*

which may suggest a geographical variation as that study was undertaken in New York.

As to the incidence of pneumo streptococci once the feared cause of many postoperative infections (Elsching & Ulbrich 1909 Gradle 1910 Imre 1911 Lowenstein 1911 v Liebermann & Lengyel 1911 Matafune & Albanese 1912 Lindner 1914 Kraupa 1914 Betti 1921 Stanka 1924 Wissmann 1924 McKee 1924 Lundsgaard 1925 Elsching 1926 Kuffler & Schneider 1926) a significant fall in frequency could be observed (Table VIII) While authors in the pre antibiotic era reported an incidence varying from 17 9 % to 46 % (Gradle 1911 Lowenstein 1911 Matafune & Albanese 1912 Lindner 1914 Kraupa 1914 Stanka 1924 Wissmann 1924 v Pellathy 1932) pneumostreptococci occur nowadays in only 2–6 % of the cases (Barfoed 1953 Smith 1954 Cason & Winkler 1954 Soudakoff 1954 Doden 1957 Glaswogger 1969 Locatcher Khorazo & Seegal 1972 Fahmy et al 1974 1975a) This is probably due to the discovery of antibiotics and better hygienic conditions However the incidence of gram negative bacilli does not seem to have changed throughout the years still being 3–8 % (Lindner 1914 Kraupa 1914 Edmund 1927 Cooper 1935 Khorazo & Thompson 1935 Barfoed 1953 Smith 1954 Cason & Winkler 1954 Soudakoff 1954 Doden 1957 Glaswogger 1969 Locatcher Khorazo & Seegal 1972 Fahmy et al 1974 1975a)

From the epidemiological point of view the relationship of the ocular flora to the nose and skin of the face seems to be of particular importance as these areas may constitute a potential source of infection per or postoperatively (Keilty 1930 Leopold & Apt 1960 Locatcher Khorazo & Gutierrez 1960 1972 Goodner 1963 Nolan 1967 Allen 1972 Fahmy et al 1975c d)

The present results demonstrated a similarity in regard to the kinds of bacteria recovered from the conjunctiva nose and face skin and a positive correlation could be found between the occurrence of some of the microorganisms A finding of *S aureus* or gram negative bacilli in one region strongly suggested their presence in the other This was further demonstrated by examining the probability of finding the same phage type complex if *S aureus* occurred simultaneously in two regions

In a series of investigations on the inhibitory properties of the normal ocular flora Halbert and co workers (1952 1953 1954 1957 1972) found that some ocular strains mainly *S albus* recovered from the eye were capable of inhibiting the growth of other microorganisms *in vitro* by producing a kind of antibiotic which was somewhat more potent than penicillin and oxytetracycline in agar plate assays Animal experiments confirmed their laboratory findings They suggested that the antibiotic properties of the normal conjunctival flora

may be of significance in the resistence of the eye to superficial bacterial infections and are probably a protective factor for this tissue against disease The data gained from the present study may support their suggestions since *S aureus* and gram negative bacilli the major offenders in ocular infections were isolated more frequently in the absence of *S albus* ($P < 0.0001$ and $P = 0.0007$) the supposed safeguard of the eye

In his thesis on *Cytology of the Conjunctival Fluid* Norn (1960) demonstrated in agreement with some other authors (Bredahl 1926 Weiss et al 1944 Duszynski 1954) that polymorphonuclear neurophils may be found in most normal conjunctivas Neutrophilia (more than 100 neutrophils per pipette specimen) was found to occur significantly more frequently in cases with infectious conjunctivitis than in normal conjunctivas (Norn 1960) This was explained by the power of leukotaxis caused by the presence of pathogenic bacteria on the conjunctiva The present study attempted to correlate the incidence of bacteria cultured from the conjunctiva with the number of neutrophils recovered from the conjunctival fluid It was quite surprising to note that the occurrence of potential pathogens i e *S aureus* gram negative bacilli and pneumostreptococci did not cause more neutrophilia than other microorganisms such as *S albus* or corynebacteria This is probably due to the above mentioned protective role of *S albus* the action of lysozyme and the content of specific antibodies in the tears (Halbert 1972) These factors were presumably successful in neutralizing the potential danger of those pathogens and prevented neutrophilic reactions

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BACTERIAL FLORA IN RELATION TO CATARACT EXTRACTION

II Peroperative Flora

BY

J A FAHMY* S MØLLER** and M WEIS BENTZON**

The peroperative flora of 499 patients undergoing cataract extraction was studied with local bacterial cultures taken at the beginning and end of surgery and compared with the preoperative flora examined previously (Fahmy et al 1975 b) on admission the day prior to surgery
The local application of a single dose of oxytetracycline – polymyxin B approximately 18 hours before surgery significantly reduced the incidence of bacteria at the time of surgery However 9[illegible] % of the conjunctivas examined immediately before operation proved to harbour one or more kinds of microorganisms Futhermore 61 % of the wound sites were found to be contaminated with bacteria at the conclusion of surgery The reasons are discussed
The origin of *Staphylococcus aureus* isolated peroperatively from the conjunctiva and wound site was studied The great majority of strains could be traced to the patient s own conjunctiva preoperatively In a few cases *S aureus* was traced to the patient s own nose skin of face or to the surgeon s nose The air of the wards and operating theatre as well as the hands and gloves of surgeons and assistant nurses apparently did not play any role as a source of *S aureus* infection

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Key words conjunctiva – wound site – flora – bacteria – cataract extraction – surgery

Previously (Fahmy et al 1975 b) the preoperative flora of 499 patients admitted for cataract extraction was studied at the time of admission to the hospital *Staphylococcus albus* was by far the most common microorganism followed by corynebacteria *Staphylococcus aureus* gram negative bacilli and streptococci None of the conjunctivas was found to be sterile

The present paper deals with the peroperative flora of the above mentioned patients The conjunctiva was examined in the operating theatre immediately before the beginning of surgery and the wound site at its termination in order to describe the ocular flora found at the time of operation and to determine the changes in the flora before and during surgery Furthermore the present study was undertaken to assess the origin of *S aureus* isolated at the time of surgery from the eye and to correlate its occurrence to some potential sources of infection known from the literature to be of particular importance i e air of operating theatre nose hands and gloves of operating surgeons and assistant nurses (Pulvertaft 1949 Allen 1959 Posner 1960 Locatcher Khorazo & Gutierrez 1960 Allen & Mangiaracine 1964 Whiston 1967 Editorial 1970 Thomsen et al 1970 Ellis 1970 Allen 1972 Locatcher Khorazo & Gutierrez 1972 Jepsen 1972 1974)

Material and Methods

The material is described in detail elsewhere (Fahmy et al 1975 b) and comprises 499 patients admitted for senile (or presenile) uncomplicated cataract extraction to the Department of Ophthalmology Kommunehospitalet Copenhagen, during the period 17 3 1972 to 16 8 1973

Sampling methods Shortly after admission cultures were taken from the conjunctiva (sample A) nose (sample B) and skin of face (sample C) as well as from the air of the wards (sample D) where the patients were admitted The methods of obtaining cultures were described previously (Fahmy et al 1975 b)

In the present study in the operating theatre and immediately before the beginning of surgery a culture was taken from the lower fornical and tarsal conjunctiva (sample E) with a dry cotton wool swab after the instillation of 1 % tetracaine hydrochloride eye drops At the end of operation another culture was obtained from the wound site (sample I) In addition cultures from the air of the operating theatre (one per operation) were recovered on 13 5 cm blood agar plates (sample F) which were placed close to the field of operation and exposed for 1 hour Furthermore finger samples (after the removal of gloves if any were used) from surgeons (sample G) and assistant nurses (sample H) were taken on 9 cm blood agar plates after the conclusion of surgery (one sample per operation) In the last 3 months of the study further samples were taken from

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BACTERIAL FLORA IN RELATION TO CATARACT EXTRACTION

II Peroperative Flora

BY

J A FAHMY* S MØLLER** and M WEIS BENTZON**

The peroperative flora of 499 patients undergoing cataract extraction was studied with local bacterial cultures taken at the beginning and end of surgery and compared with the preoperative flora examined previously (Fahmy et al 1975 b) on admission the day prior to surgery

The local application of a single dose of oxytetracycline – polymyxin B approximately 18 hours before surgery significantly reduced the incidence of bacteria at the time of surgery However 9? % of the conjunctivas examined immediately before operation proved to harbour one or more kinds of microorganisms Futhermore 61 % of the wound sites were found to be contaminated with bacteria at the conclusion of surgery The reasons are discussed

The origin of *Staphylococcus aureus* isolated peroperatively from the conjunctiva and wound site was studied The great majority of strains could be traced to the patient s own conjunctiva preoperatively In a few cases *S aureus* was traced to the patient s own nose skin of face or to the surgeon s nose The air of the wards and operating theatre as well as the hands and gloves of surgeons and assistant nurses apparently did not play any role as a source of *S aureus* infection

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positive and negative If such an independence is found, it is sufficient to investigate the two pairwise comparisons A versus E and E versus I The statistical test used for pairwise comparisons was the McNemar test

Table I

Incidence of microorganisms isolated from 499 patients on admission at the beginning and end of surgery

Microorganisms	Incidence in per cent					
	Pre operative flora		Per operative flora			
	Conjunctiva on admission (sample A)		Conjunctiva beginning of surgery (sample E)		Wound site end of surgery (sample I)	
Staphylococcus albus		95 4		87 6		56.3
Corynebacteria		44 0		3.2		2 6
Staphylococcus aureus		14 9		7 4		3 8
Streptococcus						
haemolyticus	1 8		0 2			
non haemolyticus	1 2	4 0		0 8		
faecalis	1 0		0 6			
Diplococcus pneumoniae		0 4				
Gram negative bacilli						
Escherichia coli	0 8		0 2			
Bacterium anitratum	1 4					
Enterobacter cloacea	0 8					
Proteus morgani	1 6		0 4			
Proteus vulgaris	0 4	7 8		1 6		0 8
Proteus mirabilis	1 8		1 0		0 8	
Proteus rettgeri	0 2					
Klebsiella oxytoca	0 6					
Klebsiella rhinoscleromatis	0 2					
Not identified						
Gram positive	2 4	2 8			0 8	1 0
Gram negative	0 4				0 2	
No growth		none		8 0		39 1
Not examined		0 2		0 6		0 8

Table III

Incidence and grade of contamination with *Staphylococcus albus* of the previously gloved and ungloved hands of surgeons and nurses

Operating surgeons (code)	Incidence of *Staphylococcus albus* on hands of surgeons*											
	Group XZ						Group Z					
	Sterile	Grade of growth			Not examined	Total	Sterile	Grade of growth			Not examined	Total
		+	++	+++				+	++	+++		
I	2			1	1	4	39	30	5	6		50
II	2	12	5	42		61			1	1		2
III			1			1	[illegible]	15	8	22		50
IV		3	7	70		80			1			1
V	1	1	1	5		8	6	[illegible]	8	11		62
XXXVI	2	5	[illegible]	19		31				1		1
XXXVII	14	3	[illegible]	1		20						
XXXIX	2			39	1	41						
X			1	10		11						
XXXVIII				2		2						
XXXIII				[illegible]		[illegible]			1			1
Total	[illegible]	21	[illegible]	[illegible]	2	[illegible]	[illegible]	80	[illegible]	41		[illegible]

Assistant nurses	Incidence of *Staphylococcus albus* on hands of assistant nurses**								
VI	133	104	26	21	95	48	20	0	3	71
VII	3	9	6	11	98	8	11	2	3	24
Total	136	113	32	32	313	56	31	2	6	95

* Sample G * Sample H

Croup ∖7 includes those surgeons and nurses operating u i h gloves cultures from finger samples were obtained after the removal of gloves
Group Z includes those surgeons and nurses operating *without* gloves

Table V

Number of air borne bacteria carrying particles *per* agar plate (13 5 cm) /hour and the incidence of *S aureus* streptococci and gram negative bacilli isolated from the air of wards on admission and during surgery

Colony count	Number of specimens from air	
	Wards (sample D)	Operating theatre (sample F)
None	0	0
< 10	6	0
11–20	13	16
21–40	75	131
41–80	148	268
81–160	170	72
161–320	51	1
321–640	8	0
Not examined	28	11
Total	499	499

Microorganisms	Incidence in wards and operating theatre	
Staphylococcus aureus	10	9
Streptococci	2	3
Gram negative bacilli	11	16?

Air flora of operating theatre and wards Table V shows the number of air borne bacteria carrying particles per plate and hour from the wards on admission (sample D) and from the operating theatre during the operation (sample F) The mean count per plate in sample D was about 102 colonies which assuming an average settling velocity of 20 m per hour (Thomsen et al 19 0 Jepsen 1972) and area of plate (143 cm) corresponds to 357 bacteria carrying particles per m³ The mean count in sample F was about 60 colonies per plate which corresponds to 209 bacteria carrying particles per m³ The order of magnitude of these figures was similar to that found by Jepsen (1972)

S aureus was found in 10 out of 471 (2 1 %) plates in the wards and in 9 out of 488 (1 8 %) in the operating theat e In all cases *S aureus* was bacteriophage typed and compared with those found in the eyes In no instance could a relation ship be found The same applied to gram negative bacilli *(B anitratum)* and streptococci isolated from the air of wards and operating theatre

Epidemiology of S aureus

Table VI shows the phage type complexes of *S aureus* isolated on admission (sample A) at the beginning (sample E) and at the conclusion of surgery (sample I)

Out of 37 strains recovered at the beginning of operation 34 proved to have either the same phage type complex (29 cases) or were not typable (five cases) as on admission Out of the remaining three strains one could be traced to one patient s own nose (6/42E/47/53/54/75/81/83A/85) the other to a patient s own skin of face (3A) while the third was not typable

Out of 19 strains isolated from the wound site at the end of surgery 17 had either the same phage type complex (14 cases) or were not typable (three cases) as in samples E or A One strain could be traced to the nose of a surgeon (phage type 52) The other strain which originally was not found in the patient s samples was not typable Attention must be paid to the fact that two of the nurses assisting in the operation room were carriers of non typable strains of *S aureus* However this could only suggest a source of infection but not a proof since non typable strains of *S aureus* are generally not accepted as tools in epidemiological studies

Discussion

Soon after the introduction of antiseptic and aseptic technique in general surgery the basic principles were applied to ophthalmology by Graefe in 1878 Lister s carbolic acid was applied to the eye before surgery to get it germ free

Gasparini (cited by Imre 1911) was probably the first to link the aethiology of postoperative infections occurring in ophthalmology to bacteria preexisting preoperatively on the conjunctiva

Since that time numerous ophthalmic surgeons (Elsching & Ulbrich 1909 Gradle 1910 Imre 1911 Metafune & Albanese 1912 Lindner 1914 Kraupa 1914 Stanka 1924 Lundsgaard 1925 Elsching 1926 Kuffler & Schneider 1926 Lucic 1927 Keilty 1930 Rodin 1945 Locatcher Khorazo 1953 Locatcher Khorazo & Gutierrez 1956 1960 1967 Burns & Oden 1972 Ulrich & Burton 1974) believed

Table VI

Correlation between phage type complexes of *S. aureus* isolated on admission, at the beginning and end of surgery

Code	Phage type complex	Incidence of *Staphylococcus aureus*									Not examined	Total
		Sample A	−	−	−	−	+	+	+	+		
		Sample E	−	−	+	+	−	−	+	+		
		Sample I	−	+	−	+	−	+	−	+		
	Group I											
01	80 and/or 81								1			1
02	52 and/or 29			1			4	1	4			10
03	29/52/81											
04	[illegible] 80/81											
0	Other combinations gr. I						3		1	4		8
	Group II											
06	3A				1				3			4
07	71											
08	gr. II/71											
09	Other combinations gr. II						4	1	1	1		7

	Group III										
10	49B and/or 47C										
11	A single type gr III										
12	Other combinations gr III					2		1(1)*			4
13	Gr III/83A/6557 (± 89)					1		2	1		4
14	31B/59B/6557 (± 89)										
15	Gr III/6557					1					1
16	83A/6557 (± 89)					1		1			2
17	Gr III/83A (± 89)					3		1	1		5
18	84/85/6557										
19	84 (± 89)										
20	85 (± 89)					1					1
21	84/85 (± 89)										
22	89					1		1			2
23	6557 (± 89)					1					1
24	83A								1		1
27	*Group IV*										
25	*Mixed groups*							1	2		3
28	187 and/or 94			1	4				2		7
26	Non typable undiluted		1			10	1	3(1)*	2		18
99	Not (phage) examined										79
	Total	416	2	2	0	36	3	21	14	5	499

Sample A Conjunctiva on admission
E Conjunctiva immediately before surgery
I Wound site at end of surgery

* One patient had two different types in A(12) and E(26)

in the self infection theory and several authors in the preantibiotic era (Imre 1911 Liebermann & Lengyel 1911 Löwenstein 1913 Kraupa 1914 Betti 1923 Stanka 1924 Edmund 1927 Thompson et al 1937) contributed to the diverse methods for rendering the conjunctival sac free from bacteria before operation

After the introduction of antibiotics the same line was followed by topical administration prior to ocular surgery (Dunnington & Locatcher Khorazo 1945 Hughes & Owens 1947 Dellaporta et al 1949 Locatcher Khorazo 1953 Callahan 1953) However in later years with the experience gained from general surgery some authors (Leopold 1958 Leopold & Apt 1960 Kolker et al 1967 Leopold 1971) doubted the prophylactic role of antibiotics before surgery and warned of the danger of altering the local conjunctival flora thus permitting the development of pathogens which flourish only because competitive organisms have been inhibited Consequently many surgeons today either use no antibiotic prophylaxis prior to surgery (Chalkley 1963 Hallermann 1963 Nolan 1967 Constantaras et al 1972 Jaffe 1972) or compromise by giving a single dose pre or peroperatively (Aronstam 1964 Chalkley & Schoch 1967 Cassady 1967 Kolker et al 1967 Hughes 1971 McPherson 1971 Boyed 1972 Christy & Lall 1973)

The latter policy is practiced in our department Accordingly and on the basis of the present results (Table I) 92 % of the eyes examined immediately before surgery proved to harbour one or more kinds of bacteria Of these 11 % belonged to the so called potential pathogens i e were *S aureus* streptococci or gram negative bacilli At the conclusion of surgery bacteria were cultured from the wound site in 61 % of the cases Of these 8 % were either *S aureus* or gram negative bacilli

These results are in agreement with almost all other studies on the peroperative flora where no or else a single dose of antibiotics dose was administrated pre operatively (Chalkley 1963 McMeel & Wagner 1965 McMeel 1965 Lincoff et al 1965 Nolan 1967 Makley & Suie 1971) and are explicable by inadequate prophylactic treatment

The fact that the wound site was found to be less contaminated with bacteria at the end of surgery than the conjunctival cul de sac immediately before operation is explained by the topographical distribution of bacteria on the conjunctiva Elsewhere (Fahmy et al 1974) it has been demonstrated that the bulbar conjunctiva a priori harboured less bacteria than the lower fornical conjunctiva. Furthermore no anaesthetic eye drops were used immediately before obtaining cultures from the wound site while tetracaine eye drops were installed before taking samples immediately prior to surgery Fahmy et al (1975 a) showed that the instillation of eye drops in connection with obtaining cultures significantly increased the recovery rate of *S albus*

According to the present study a single dose of oxytetracycline polymyxin B 18 hours before surgery was able to reduce significantly ($P < 0\ 0001$) the incidence of all types of bacteria at the time of operation A longer and more intensive preoperative treatment to render the conjunctiva free from bacteria seems to be necessary In the preantibiotic era several days of treatment were needed to get the conjunctiva sterile (Liebermann & Lengyel 1911 Lowenstein 1911 Lindner 1914 Kraupa 1914 Betti 1921 Edmund 1927) When antibiotics were used topically for many days prior to surgery Locatcher Khorazo & Gutierrez (1972) found that *S aureus* was usually eliminated in 2–3 days while gram negative bacilli persisted for as long as 7 days

The relationship of postoperative infections to preoperative treatment is discussed elsewhere (Fahmy 1975)

One of the important objects of the present investigation was to study and analyse the epidemiology of peroperative flora i e to assess the origin of bacteria cultured at the time of surgery *S aureus* was chosen as an index for several reasons Primarily because it is generally considered to be the major offender in postoperative infections in ophthalmology and because it occurs relatively infrequently in normal eyes and surgical environment and finally because of its suitability for bacteriophage typing

Out of 37 strains isolated from the conjunctiva immediately before surgery 34 proved to have either the same phage type complex or were not typable as on admission Two strains could be traced to the patient s own nose and skin of face In only one case could the source of a strain not be found since it was not typable At the conclusion of surgery 19 strains were cultured from the wound site 17 of these had either the same pattern or were not typable as at the beginning of surgery or on admission One strain was traced to the nose of a surgeon while another one was not typable

On the basis of the above mentioned facts it may be concluded that the source of *S aureus* isolated at the time of surgery is predominantly the patient s own conjunctiva In a few cases infection may occur from the patient s own nose skin of face or from the surgeon s nose

The role of the air of wards and operating theatre as a potential source of *S aureus* infection seems to be insignificant primarily because of its low contamination grade with *S aureus* The same may be said of hands and gloves of surgeons While in the United States the use of gloves during ophthalmic surgery is obligatory (Jaffe 1972) there are many surgeons in Europe who operate without (Lund et al 1974) According to the present study both habits seem to have no influence on the peroperative occurrence of *S aureus*

Ulbrich M R A & Burton T C (1974) Infections following scleral buckling proce dures *Arch Ophthal* 92 213-215
Whiston G J (1967) Review of postoperative endophthalmitis *Canad J Ophthal* 2 63-69

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TRANSACTIONS OF THE SWEDISH OPHTHALMOLOGICAL SOCIETY

EDITED BY

GUNNAR LENNERSTRAND

Meeting in Linkoping April 27 1974

U Axelsson *Atypical band keratopathy and pilocarpine treatment*

Atypical band keratopathy was denoted by Kennedy et al (1971) as a keratopathy characterized by subepithelial deposition of calcium and iron and seen in glaucoma patients on long term miotic therapy In a typical case the changes start paracentrally in the lower and nasal portion of the cornea in contradistinction to the true band keratopathy where the changes start in the periphery both nasally and temporally Kennedy et al recommended irrigation with a 0 02 M solution of EDTA as a simple and successful treatment.

At the Eye Clinic of Sabbatsberg Hospital Stockholm in 1969 a man with a narrow angle glaucoma was observed who after 6 year s treatment with pilocarpine – the last 4 of these with pilocarpine in methylcellulose – developed a central subepithelial opacification in both corneas Sector shaped bilateral iridectomy resulted in improvment of vision and normalisation of tension After cessation of the pilocarpine therapy the corneal opacities remained unchanged

During the last year another eight patients have been observed in whom 12 eyes on pilocarpine therapy have shown this atypical band keratopathy The treatment time has varied from 15 months up to 11 years Out of these eight patients seven have been on pilocarpine in methylcellulose for at least 10 months In five patients pilocarpine was the only treatment given whereas in three epinephrine preparations and/or carbonic anhydrase inhibitors were added to the regimen The severity of the glaucoma did not seem to influence the development and progression of the deposition.

In seven patients (10 eyes) EDTA irrigation was undertaken in every case with good result In some cases a dramatic improvment of the visual acuity was noticed In two preexisting nasal field defects disappeared

In rare cases atypical band keratopathy can be seen in eyes never treated with miotics

Its more frequent appearance in miotic treated patients who undoubtedly co[illegible] a minority of the population seems to indicate that the deposition in some way is caused by the treatment Whether this is achieved by the miotic agent or its vehicles is so far not known

References

Kennedy R E Roca P D & Landers P H (1971) Atypical band keratopathy in glaucomatous patients *Amer J Ophthal* 72 917

B Ehinger & E Palm *Experimental iris sutures in the monkey*

Artificial iris wounds were sutured with 30 μ monofilament nylon sutures in 15 monkey eyes and studied histologically at different intervals up to 6 months

The monkey iris does not form any scars When two wound lips are apposed by exact suturing they join The mechanical strength of the union is yet unknown The iris reacts to the operation but not enough to preclude the procedure There has been no adverse late reaction to the suture material for at least 6 months after the operation Published 1973 *Acta ophthal (Kbh)* 51 853–860

E Schyberg *On the diagnosis of unilateral exophthalmos with special reference to fine needle biopsy*

Expanding lesions of the orbit causing unilateral exopthalmos often present difficult clinical problems It is of utmost importance to get a correct diagnosis quickly since malignant tumours require early treatment and unnecessary surgical procedures on benign tumours should be avoided

During 1973 we examined 10 cases of unilateral exophthalmos In six cases the etiology was tumour one case was in connection with hyperthyreosis and three cases were unexplained (also thyroid tests including i v TRH stimulation test were negative)

Two of the six tumours (meningeomas with orbital and intracranial expansion) were diagnosed by carotid angiography the remaining four by fine needle biopsy and cytological examination (one plasmocytoma one haemangiopericytoma two meningeomas) These four tumours could be well localized by ultrasound whereas phlebography and scintigrams were less helpful in localizing the tumours

In earlier studies fine needle biopsy has not been considered the method of choice for diagnosing orbital tumours However in the present study it has been shown that a careful localization of the tumour followed by adequate fine needle biopsy and cytological examination are good alternatives to and often even superior to other methods

The procedure is easy to perform causes little or no discomfort to the patient and the risk of spreading of the tumour seems to be small

K O Skoog & S E G Nilsson *The c wave of the human electroretinogram*

Until now only the comparatively fast *a* and *b* waves which originate in the receptor layer and in the inner nuclear layer have been used in the clinical ERG procedure However since damage of the pigment epithelium e g drug induced may secondarily involve the neuroretina it should be of clinical interest to find a method to record changes in the ERG reflecting early pigment epithelial injury This requires a quantitative registration of the human c wave which according to intracellular recordings on animals has been shown to originate in the pigment epithelium Our present method is based on the work of Knave Nilsson & Lunt (1973) with several modifications It

includes the use of special calomel electrodes d c amplification and averaging technique and it gives stable and reproducible recordings of the human *c* wave without the aid of general anaesthesia With this method the *c* wave amplitude was studied at stimulus intensities ranging from 3.5 to 5.5 relative log units above the *b* wave threshold. The lowest intensity eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log relative intensity 0 With a stimulus duration of one sec a linear relationship between log stimulus intensity and the *c* wave amplitude was found within the investigated range of intensities (2 log units) It was difficult to measure the *c* wave at lower intensities and higher intensities often provoked disturbing eye movements Long term experiments with a stimulus interval of 30 sec showed a damped cyclic change of the *c* wave amplitude with a frequency of about ?/hour These results should be of importance when developing a clinical method The method has to be further standardized, and then a sufficiently large number of registrations on normal volunteers must be performed

Publ (1974) *Acta ophthal (Kbh) 5?* 759–773 and 904–91?

I E Wålinder *Band keratopathy in glaucomatous patients*

Band keratopathy is usually associated with chronic eye disease e g uveitis or systemic disease with hypercalcemia The opacity consists mainly of subepithelial calcium deposits Kennedy et al (1971) (*Amer J Ophthal* 7? 917) described a band keratopathy in eyes with glaucoma and they mentioned a simple treatment.

In the present study the eyes of 500 patients with glaucoma were examined in the microscope and a bilateral band keratopathy was found in six cases three of them had very small opacities Thus the incidence is low in this study about 1 %

The total series consists of 10 well controlled glaucomatous patients with bilateral band keratopathy Fine grey or grey brown granular subepithelial deposits were observed in the paracentral or nasal parts of the cornea Thirteen eyes were treated as previously described the epithelium was removed over the involved area and 15 ml 0 02 M solution of EDTA was dropped continuously on the cornea for 15 min During this treatment the opacities disappeared completely in almost all eyes Nine eyes were then controlled for 1 year and despite continued miotic treatment there were no recurrences

The patients had no chronic eye disease except the glaucoma and there were no hypercalcemias The question then arises as to whether the glaucoma or the treatment can be associated with the keratopathy One patient had keratopathy when the glaucoma was discovered nine patients had been treated with miotics between 0 5 and 20 years before the opacities were observed, four of them had used pilocarpine only five had used both pilocarpine and other miotics adrenaline or acetazolamide The miotic treatment may thus be of importance in the development of band keratopathy but further studies are required to settle the question

Meeting in Stockholm November 30 1974

P Enoksson & B Fristrom *Double point test with the Goldmann perimeter*

Two prisms (base out) attached to the projector of the Goldmann perimeter made it possible to have simultaneous projection of two spots of light e g at either side of the vertical meridian Results were demonstrated with the technique used as in colour saturation test in a case of chiasmal lesion with partial temporal hemianopia and as a new method for recording inattention hemianopia in a patient with a right cerebral lesion kinetic perimetry showed normal visual fields but the double point test showed left homonymous hemianopia The technique was demonstrated at the meeting of the Swedish Ophthalmological Society in Gallivare on August 31 1974

A Hedin *Eyelid spring for correction of lagophthalmos*

Lagophthalmos due to orbicularis palsy gives ocular irritation is cosmetically annoying and may lead to corneal ulcers When nerve anastomosis is impossible or does not lead to sufficient orbicularis function palliative methods are necessary Among the current methods are tarsorrhaphy muscle transplantation lid loading implantation of a silicone thread and the use of permanent magnets The eyelid spring was presented by Morel Fatio and Lalardrie in 1962 This spring of 0.3 mm stainless steel is fixed to the orbital margin under the brow and in the outer canthus and its free arm is sutured to the anterior surface of the tarsus Eyelid opening and closure is now obtained by the opposite action of the spring and the levator The technique is simple if one has some experience in handling a pair of tongspand steel wires The spring is fashioned exactly to the patient s eyelids It is inserted from the lateral side with the aid of two needles and the free ends are cut and bent to a loop The two marginal loops are fixed to the periosteum with non absorbable sutures and the lower loop is sutured to the tarsus with 7–0 silk The patient is now asked to blink and the tension adjusted

So far eight patients with lagophthalmos have been operated In one the spring was removed after 2 months owing to a persistent low grade wound infection. The other seven have perfect toleration and all patients but one are without any ocular irritation or require treatment In two cases the spring tension had to be adjusted Eyelid edema often persists for many weeks but complications are otherwise few In general the paralysed lid opens a few millimeters less than the other lid and the rima is not completely closed often due to a coexisting lower lid ectropion In summary the eyelid spring is a simple palliative method for correction of lagophthalmos and the results are in most cases satisfactory

T Jerndal *Inherited malformations of the anterior ocular segment*

The complicated pattern of the fetal development of the anterior ocular segment will often result in a disturbed differentiation and maldevelopment Both sporadic and genetic malformations are found and the heredity pattern is autosomal dominant Among the inherited malformations there is a number of characteristic structural defects which may appear isolated or in various combinations

One genetic maldevelopment may often be constant in its localization, e g in the iris but quite inconstant in its severity or expressivity This variability of expressivity is less well known to the clinician but nevertheless is of the greatest importance for the

evaluation of the heredity treatment and prognosis

The actual malformations are microcornea macrocornea iris hypoplasia iris hyper plasia aniridia lens luxation and goniodysgenesis

The detection of angle maldevelopment or goniodysgenesis is particularly important since it implies a predisposition for glaucoma – early or late congenital glaucoma

P Jahnberg & A Bjork *Retinal dystrophy combined with alopecia*
(To be published in *Acta ophthal (Kbh)*

S E G Nilsson B Knave and H Persson *Studies on the structure and function of the sheep retina after administration of sodium iodate*

Earlier investigations generally favoured the notion that the slow *c* wave of the electroretinogram originates in the pigment epithelium The present study aims at further elucidating this problem

Method I v injection of 30 mg per kg b wt. sodium iodate given to sheep D C registration of the ERG at the time of administration of sodium iodate and 3 days after such administration. Perfusion fixation and preparation for electron microscopy of the retina and the pigment epithelium 2.5 hours as well as 3 days after injection of sodium iodate

Results I v injection of sodium iodate immediately abolished the *c* wave of the ERG The *c* wave was replaced by the so called remnant negativity No decrease was seen in *b* wave and to a lesser extent in *a* wave amplitudes until about 100 min later The ultrastructural investigation of eyes fixed 2 5 hours after the injection showed a dramatically changed pigment epithelium with fractured membranes swollen and ruptured mitochondria myelin figures and aggregated microsomes The rods and cones were entirely undamaged and also the other parts of the retina were mainly normal

Three days after the administration of sodium iodate *a* and *b* waves were still seen although they were clearly subnormal The *c* wave was missing The electron microscopic study demonstrated that the pigment epithelium was changed in the same way as when fixed 2 5 hours after the injection However now also the receptor cells were markedly damaged with vesiculated and disorganized outer segments and vacuolized inner segments The Muller cells the bipolar cells and the neuronal processes of the inner plexiform layer also showed obvious signs of injury at the ultrastructural level

Conclusion Administration of sodium iodate was immediately followed by an abolishment of the *c* wave of the ERG The earliest ultrastructural changes were localized to the pigment epithelium which was seriously damaged Only later could one observe alterations of the *a* and *b* waves of the ERG and electron microscopic signs of injury to the receptor cells and other parts of the retina The results give strong support to the notion that the *c* wave is generated in the pigment epithelium

K O Skoog & S E G Nilsson *Effects of ethyl alcohol on the c wave of the human electroretinogram*

A recently described method permits for the first time stable and reproducible registrations of the *c* wave of the human electroretinogram without the aid of general anaesthesia With this procedure it has been possible to demonstrate slow (2/hour) amplitude oscillations of the human *c* wave Also the effect of various drugs and other substances on the *c* wave can be studied without any interference from general anaesthetics Animal experiments have shown that a number of substances with known

ocular toxicity influence the *c* wave As an introduction to a series of experiments with different drugs the *c* waves of 10 volunteers were studied before and after oral administration of ethanol

Method With special very stable calomel electrodes d c amplication and an electronic averaging technique d c ERGs including the *c* wave were recorded twice a minute for at least 1 hour An amount of 0 4 g ethyl alcohol/kg body weight was given orally at different times after the beginning of the experiment

Results Alcohol caused a marked increase in amplitude of the *c* wave oscillations and also raised their mean level A first peak was reached 10–15 min after the intake of ethanol Also the *b* wave amplitude increased while there was no significant change in the *a* wave

Conclusion Even a small dose of ethyl alcohol influences the human *c* wave dramatically The present results encourage further investigations concerning the action of drugs on the *c* wave as a part of a study of the ocular toxicity of various substances
Publ (1974) *Acta ophthal (Kbh)* 52 913–923

K. Wettrell & M Pandolfi *Effect of oral administration of various beta blocking agents on the intraocular pressure in healthy vonluteers*
(To be published in *Ophthalmologica)*

TRANSACTIONS OF THE DANISH OPHTHALMOLOGICAL SOCIETY 1973–1974

BY

ERIK SCHERFIG Secretary

454th Meeting October 6 1973 in Copenhagen (Rigshospitalet Blegdamsvej)

General Meeting with *Ernst Goldschmidt* in the Chair

One of the subjects the members discussed was the introduction of an authorization for opticians and *J Edmund* was given a mandate to apply to the Ministry of the Interior concerning this problem

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455th Meeting October 6 1973 in Copenhagen (Rigshospitalet Blegdamsvej)

S Medgyesi (guest) *Periorbital reconstruction*

In the ocular area reconstruction tasks are numerous A systematic review cannot be given here but a few of the principles we have found suitable for the most common ones are mentioned Loss of skin alone is replaced with grafts of partial thickness skin to the upper eyelids and full thickness skin to the lower eyelids As partial thickness skin has a tendency to shrink it is important to over correct the upper eyelid which is stretched and fixed to the cheek until the first dressing is renewed For skin grafting to the lower eyelid skin from the upper eyelid or a full thickness graft from the retroauricular region is used as the colour of the skin from this site matches well with the ocular surroundings Ectropion after the removal of xanthelasmas and the like may be avoided

by primary skin grafting Particular attention was paid to eyelid burns In the event of full thickness loss it is important to graft soon. If not shrinkage will prevent closure of the eye resulting in desiccation and keratitis In certain posttraumatic cases it has been necessary to replace a lacking lower tarsal cartilage We have obtained acceptable results by grafting with cartilage from the ear However it may be difficult to find the optimal shape and thickness In cases of major total defects of the lower eyelid, the conjunctiva and tarsus from the upper eyelid should be mobilized so that the eyeball is covered and thereafter skin from the upper eyelid is carried down as a bridge flap After 4 weeks the tarsorrhaphy is opened. In cases where the entire lower eyelid is absent we have obtained good results with the method of Mustarde in which a rotation flap from the preauricular region is lined with a free cartilage mucosa graft taken from the nasal septum Obstruction of the lacrimal ducts has been satisfactorily repaired by conjunctivo rhinostomy with the insertion of a glass tube as advocated by Lester Jones An important condition for good function is that the nasal cavity is not narrowed, e g by septal deviation The most difficult part of this operation is to place the end of the tube free in the nose The procedures described were illustrated by examples

Discussion *E Gregersen Ry Andersen E Goldschmidt P Brændstrup J Edmund*

M Pers *The contracted orbit*

A special problem is posed by the empty orbit which will not house and retain a prosthesis The presence of some conjunctiva is an advantage, as the smooth lining of the eyelids is necessary for their movement in relation to the prosthesis The floor of the orbit may consist of a partial thickness graft placed as an inlay Transplantation of oral mucosa has not been successful in our hands but skin and conjunctiva are compatible – at least after a couple of years have elapsed Usually the less there is of the conjunctiva the less there remains of the eyelids and it is difficult to avoid the staring look But even though the eyelids are completely absent acceptable results may be attained by alternately adding free skin grafts to the inside and outside of new eyelids. Infants with microphthalmy and anophthalmy are best treated by expansion as used in the Institute for the Blind Once operation has been performed it is very difficult to form a sufficiently large cavity as the small eyelids become shrunken and lax making it difficult to retain a prosthesis As the bony orbit cannot hold the prosthesis the latter has to be placed anteriorly to it and we have had to reinforce the lower eyelid with temporal fascia to secure a deep inferior fornix Generally in grafting skin to empty orbits the prosthesis used in the operation should not be spherical but lenticular with a concave posterior aspect Furthermore it is an advantage to fix it with a steel wire to the supra and infraorbital margins and leave it untouched for at least 6 weeks behind a tarsorrhaphy with small openings medially and laterally for irrigation After total evacuation of the orbit the cavity may be lined with a free graft but this leaves a greatly disfigured cavity which has to be covered with an exoprosthesis The result is less disfiguring if the cavity is filled with temporal muscle introduced through an opening in the lateral orbital wall If the muscle flap is covered with a pedicled flap from the forehead it may later be possible to insert a prosthesis surrounded by free skin graft Finally the palpebral fissure is opened so that the patient can be fitted with a proper ocular prosthesis

The various conditions were illustrated by examples

Discussion *H Skydsgard E Goldschmidt*

E Dreisler and J Edmund *Orbital implantation prosthesis*

With an orbital implantation prosthesis it is possible to lend a glass eye some mobility and to place it at a more normal level This does not lead to an abnormal supratarsal sulcus and after enucleation in children the growth of the facial bones will be more normal The implantation materials polystan in a wire ball and autologous subcutaneous fatty tissue were compared

In the Eye Department of Rigshospitalet University of Copenhagen, 35 polystan balls have been inserted in the course of the past 6 years The case notes have been perused In two patients the ball has worked its way through the conjunctiva without any signs of infection In one patient the conjunctiva and Tenon s capsule has been resutured three times and in the other patient resuturing has been done once Only in one patient has the ball been expelled This happened 10 months after the enucleation Ten months later the orbit was not yet dry All the other patients have had no problems during follow up periods from 3 months to 4 1/2 years mean 21 months Secondary implantation in one case, 9 years after the enucleation has not given rise to problems in 4 1/2 years The cosmetic results are good as assessed by examination of some of the patients

In the Eye Department at Nykobing Falster 24 fatty tissue grafts have been inserted in the course of 15 years None has been expelled or has required resuturing Two patients have had complications in the wound at the donor site One developed a haematoma in the subcutaneous tissue followed by infection and abscess formation The other one developed simple rupture of the wound with normal healing after secondary suture In an attempt to assess the shrinkage of the implant 20 patients – with a follow up period exceeding 5 years mean 11 years – were asked to attend for further examination One had died 3 years after the enucleation of a choroid melanoma without invasion of the sclera or optic nerve Of the remaining 19 the 14 seen at follow up had a convex conjunctival surface as a base for the prosthesis In half of these patients there had been some shrinkage and the surface of the implant was not uniform, but in all cases the cosmetic result was better than that usually found many years after enucleation without implantation

The paper was illustrated by slides of the patients and implantation materials

Discussion *S Medgyesi H Skydsgård J Edmund M Pers H Fledelius E Goldschmidt*

D Riise (guest) *Reconstruction of the eyelids in palpebral tumours*

Two methods for reconstructing the eyelids after removal of palpebral carcinomas were described

Examples of transferring the tarsal conjunctiva and skin from one eyelid to another were given

Discussion *N C Petersen S Ry Andersen*

J Edmund *Surgical treatment of blepharoptosis*

A material of 100 cases was submitted The great majority of cases viz 95% were congenital ptosis and 5% pseudoptosis Half the congenital cases were simple ptosis the other half combined predominantly with concomitant squinting abnormal ocular movements or malformations of the eyelids Among the acquired cases the great majority were myogenic due to juvenile myopathy or senile myatonia

A total of 122 operations were performed Of them 105 consisted in resection of the levator muscle of the upper eyelid The result of the operation had to be called unquestionably poor in only three cases in two of which the ptosis was combined with ophthalmoplegia or synkinesis Among the remaining cases 77 showed optimal and 49 satisfactory results

It is concluded that even with a greatly reduced levator function shortening of the levator should be the first operation to be used as satisfactory results are often obtained by extensive shortening even in cases with practically abolished levator function

Discussion *F Westerlund K Dreisler N Ehlers N Willumsen P Brændstrup*

E Goldschmidt *Scleromalacia perforans treated with a fascia lata graft*

A case of scleromalacia perforans in a 60 year old patient with a long history of rheumatoid arthritis was reported

The perforating scleromalacia had resulted in a pea sized staphyloma nasally in the left eye between the cornea and the insertion of the rectus medialis The entire sclera was greatly thinned and a circular strip of fascia lata was placed and fixed beneath the conjunctiva

There was very little postoperative reaction and visual function is well preserved

Autografts and in particular fascia lata are recommended as a material for scleral grafting

Discussion *S Ry Andersen V Ohrt*

456th Meeting November 3 1973 in Odense (Odense Hospital)

P Brændstrup *Cataract operations in the Eye Department of the Copenhagen City Hospital during the past 20 years Increasing number – extended indications*

During the period from the late 40 s to the early 70 s the number of operations for senile cataract in the Eye Department of the Copenhagen City Hospital has increased from about 100 to between 500 and 600 *per annum* There is little tendency to a flattening of the curve which has been markedly rising especially in recent years

The following explanations for the increasing number of cataract operations were advanced

1 A 60 % increase in the potential cataract clientele due to the altered age distribution of the Copenhagen population
2 Extended indications – ever more operations are being performed for minor cataract induced visual handicaps
3 Popularization of and increased motivation for cataract operation among the elderly

It was substantiated that the increased number is not due to a reduction in the number of Copenhageners having cataract operations in the Eye Departments of Rigshospitalet

The results are based upon the perusal of case notes for 330 consecutive cases undergoing operation in the Eye Department of the Copenhagen City Hospital during the years 1947 1948 1949 and 1950 and 100 cases from the years 1969 and 19 0 plus the corresponding case notes from the Eye Departments of Rigshospitalet for the years 1948 1949 1959 and 1969

M S Norn *Corneal thickness after cataract extraction*

Published in 1973 as Pachometric study on the influence of corneal endothelial vital staining – Corneal thickness after cataract extraction studied by vital staining with trypan blue. *(Acta ophthal (Kbh) 51 6 9–696)*

J A Fahmy *On the preoperative flora of the eye – Bacteriological analysis of 5 0 cataract patients*

Preliminary report (To be published in *Acta ophthal (Kbh))*

Th Rosenberg *Cystoid macular oedema following cataract surgery*

Macular oedema following an otherwise uncomplicated cataract extraction has been reported in 2%–70% of cases The proximate pathogenetic factor is a raised permeability of the perifoveal capillaries

In some patients the oedema is permanent and irreversible damage to central vision may occur

The clinical appearances including fluorescein angiographic findings were described and characteristic cases were demonstrated.

Discussion on the first four papers *A A Dresler E Gregersen Th Rosenberg H Skydsgard J Edmund E Goldschmidt P Brandstrup N Ehlers M S Norn Viggo Jensen M Hesse J A Fahmy*

A M Land *Chandler's syndrome*

In 1956 Chandler described a syndrome which he interpreted as a kind of essential iridic atrophy but exhibiting features differing from this disease

Chandler's syndrome is characterized by unilateral occurrence mild atrophy of the iridic stroma without involvement of the pigmented layer peripheral anterior synechiae to and anterior to Schwalbe's line a normal or slightly deformed pupil normal to moderately elevated intraocular tension without involvement of the optic disc or visual field dystrophy of the corneal endothelium leading to corneal oedema at an intraocular tension which would not normally affect the cornea

The history is characterized by complaints of unilateral visual impairment and rainbow vision

The aetiology is unknown

Treatment is directed at the corneal oedema Medication in the form of miotics adrenaline and carboanhydrase inhibitors is used to the extent needed to lower the intraocular tension to below the critical level for the eye concerned If this does not prove sufficient there is an indication for fistulizing operation

A case was reported in a 44 year-old woman who has been followed up for 10 years in the Eye Department of the Odense Hospital and whose history is compatible with Chandler's syndrome

Discussion *E Gregersen M Warburg V A Jensen V Dreyer M Hesse*

M Verdich and P M Møller *A case of Kearns syndrome* (Demonstrated by a film)

This syndrome comprises external ophthalmoplegia retinal pigment degeneration and complete heart block

It was described first by T P Kearns in 1958 and since then several cases have been reported

The present patient was a boy of 17 Apart from the retinal pigment degeneration external ophthalmoplegia and heart block he also had ataxia impaired hearing and increased protein in the spinal fluid

In describing the ocular findings special emphasis was placed on the benign form of retinal pigment degeneration with a good visual function unlike typical retinitis pigmentosa

The case was illustrated by a film

In the discussion of this case it was emphasized that others have conclud d that the ophthalmoplegia is of myogenic nature

Treatment aims primarily at the cardiac condition consisting in perm ent pace maker treatment

E Goldschmidt and H Schmidt *Syphilitic iritis with spirochaetae in the aqueous humour*

A case of secondary syphilis with iritis in which dark field examination of the aqueous humour revealed motile treponemas
(Published 1972 in *Brit J vener Dis* 48 400–401)

457th Meeting December 1 1973 in Copenhagen
(Rigshospitalet Blegdamsvej)

Conference on Clinical Pathology arranged by the Institute of Ophthalmic Pathology University of Copenhagen under the auspices of
S Ry Andersen O A Jensen and J Kleener

458th Meeting February 16 1974 in Copenhagen
(Rigshospitalet Blegdamsvej)

M S Norn *Scleral plaques*

Scleral plaques consist in sharply demarcated transparency of the superficial scleral layer anteriorly to the attachment of the horizontal ocular muscles

Scleral plaques are growing increasingly common among normal elderly people In an examination of 1 086 persons I found 3 ‰ in 60 year-olds 10 ‰ in 70 year olds and 25 % in those of 80 or over

My total material comprises 111 cases which considerably exceeds what has been reported in the world s literature (75 cases in all)

Plaques are most often oval their long axis vertical (65 %) or circular (97 ‰) and as a rule they are localized anteriorly to the medial ocular muscles

References

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Norn M S (1974) Scleral plaques I Incidence and morphology *Acta ophthal (Kbh) 52* 96–106

Norn M S (1974) Scleral plaques II Follow up Cause *Acta ophthal (Kbh) 52* 512–520

Discussion *V Dreyer S Ry Andersen E Vesterdal J Edmund*

N Ehlers F Kissmeyer Nielsen, K. E. Kjerbye and L U Lamm *HL A types in uveitis* Published in *Lancet* January 19th 1974

Discussion *M S Norn V Dreyer M Warburg*

J A Fahmy *Bacterial flora of the nose Sensitivity findings in the staff of an Eye Department*

To be published in *Acta ophthal (Kbh)*

Sv E Simonsen *Stereophotography of the fundus*

The value of stereophotos in elucidating retinal lesions was elucidated by clinical photos

Discussion *P Brændstrup H W Larsen Kristianson H J Jensen*

M Warburg *Prevention of hereditary diseases of the eye*

The necessity of sufficient genetic advisory activity was pointed out, and a number of clinical examples were submitted

Discussion *E Gregersen*

E Godtfredsen *Photobiological basis of visual perception*

Recent advances within the physical chemical basic sciences have also benefited pho tobiology especially in the field in which the interaction of light energy with our visual apparatus results in visual perception The quantum wave mechanics have ex plained the nature of light The views on the structure and function of the visual apparatus have been radically altered by electron microscopy micro spectrophotometry advanced neurophysiology and biochemical analytical techniques using isotopes and chromatography

Keeping abreast of the numerous advances may be quite a burden, and perhaps the following survey is motivated The nature of light was placed in historical relief by the ancient Greek philosophers of nature Pythagoras (emission theory) and Democritus After classical physics was founded in the 17th century the most important optic

discoveries were by Newton (colour dispersion of light) Huyghens wave theory and Romers ingenious measurement of the speed – or hesitation – of light Shortly after 1800 Young demonstrated the interference of light showing that light is a wave like water and later the polarization of light Maxwell placed light as an electromagnetic wave in the spectrum which was completed during the subsequent years The 0th century brought Planck s quantum theory and on the basis of photoelectric aspects Einstein advanced his light theory light as quanta Bohr s atomic theory (1913) reconciled the wave and quantum theories complementary concepts

The net gain to biology and ophthalmology respectively of modern physics is in part practical (the numerous new light sources up to laser and fibre light) in part theoretical viz insight into the energy substance interaction

The light quantum meets the electron the dynamic element of substance and after absorption a varying conversion of energy takes place e g to heat fluorescence photo electricity or – what is relevant in our field – the photochemical processes which we encounter in our photoreceptors

The retinal photoreceptors are furnished with lamellae with visual pigments – of which rhodopsin is a paradigm – made up of a protein part (about 50 000 Dalton) and a prosthetic lipid retinal derived from vitamin A = retinol On exposure to light retinol is converted from the cis to the trans form a stereo geometrical molecular change and dissociated from the protein part This initiates the receptor potential which is transformed to action potential by the complicated pattern of axons and appurtenant synapses with varying neurotransmitters (dopamine acetylcholine GABA etc) carrying the impulse to the visual cortex

It was concluded that visual perception is one of the great wonders of life whose exploration has always been and still remains a challenge to the best brains of natural science to whom we owe what we know and understand to day

K Work *Photocoagulation – Technique*

The physical basis of photocoagulation was briefly described partly the therapeutic technique and in particular the Zeiss xenon photocoagulator

J Edmund *Laser coagulation*

A clinical material from Rigshospitalet treated by laser coagulation was submitted

N Bulow *The healing process in experimental laser lesions in the retinal pigmentary layer and sensory cell layer Light and electron microscopic studies*

In monkey eyes a series of perifoveal injuries were induced by Ruby laser These injuries were restricted to the pigmentary and sensory cell layers of the retina At the time of fixation the ages of the injuries were 0 8 16 24 32 40 48 56 64 ?? hours and 3 4 6 9 13 18 25 and 36 days The lesions were isolated each in a block and cut at right angles to the retinal plane One half of each lesion was used for light microscopy of 1 μm serial sections the other half for electron microscopy of selected areas

Main approach

The not entirely fresh lesions involving the outer layers of the retina often showed cells bearing some resemblance to the cells of the retinal pigmentary epithelium but without showing all the characteristics of the pigmentary epithelium Two groups were discerned

1 Cells very similar to the cells of the pigmentary epithelium and often called proliferated pigmentary epithelium or displaced pigmentary epithelium according to the genesis which is most likely in the case concerned The formation and development of these cells have not been described

2 Cells bearing little resemblance to pigmentary epithelial cells but which apparently are able to phagocytize and break down injured tissue, and are accordingly most often called macrophages Some authors have pointed out that such cells may perhaps be formed by the pigmentary epithelium eith r by proliferation or by modification of cells which are imagined to detach themselves from Bruch s membrane in order to migrate into the lesion Some studies have been centred particularly on this problem which so far remains unelucidated.

Main results

From the study it may be concluded that in the course of the first 3 days after the induction of an experimental injury to the pigmentary epithelium and sensory cell layer of the retina a proliferation of pigmentary epithelial cells occurs around the lesion. The cells thus formed invade the lesion where they develop partly into a layer of cells resembling pigmentary epithelium along Bruch s membrane and partly into migrating phagocytizing cells which break down the injured tissue

Four days after the induction of the lesion the injured tissue has been broken down and the area is filled with the newly formed cells Thereafter the scar tissue is consolidated and at the same time the previously phagocytizing cells exhibit a considerable melanogenetic activity

The substantiation is based partly on the absence of invasion by cells from the choroid or retinal vessels and partly on a series of photographs which may be interpreted as different phases of the process described.

Th Rosenberg *Fluorescein angiography – Technique and indications*

After a brief introduction on the possibilities and limitations of angiography a series of slides was shown

In this connection, the angiographic characteristics of a number of vascular inflammatory and degenerative diseases of the ocular fundus were described.

J Edmund and K Work *Indications for and use of photocoagulation elucidated by a 10 year material from Pigshospitalet University of Copenhagen*

Photocoagulation in diabetic retinopathy A material of 174 patients treated over a 10 year period with coagulation for diabetic retinopathy was presented

Of these patients 92 were males and 82 females A total of 74 had been in the age range 0–20 years when diabetes was diagnosed 38 between 21 and 40 years The remainder were 41 years or over when the disease was detected 140 of the patients were on insulin, whereas 34 were being treated merely by diet or oral antidiabetic agents

Prior to the photocoagulation 150 or more than 86 2 % of the patients had a visual acuity of 6/18 or more in the better eye while 14 (8 1 %) had 6/24 to 6/60 in the better eye and 10 (5 8 %) had less than 6/60 in the better eye

In the poorer eye the visual acuity was 6/18 or more in 83 patients (47 7 %) 6/24 to 6 60 in 35 patients (20 1 %) and less than 6/60 in 56 (32 2 %) of the patients

Simple diabetic retinopathy was found in 33 patients (19 0 %) in the better eye and in 14 (8 1 %) in the poorer eye The others had proliferative diabet c retinopathy

The International Symposium on Fluorescein Angiography

will be held in Ghent Belgium from 28 March to 1 April 1976 The main topics will be fluorescein angiography of pigment epithelium choroid and retinal periphery *Sessions* will also be devoted to instrumentation and techniques ocular *hemodynamics* and diabetes Each session will be introduced by invited lecturers *President* *Professor* J Francois Ghent Secretary of the Committee Dr J J De Laey *University of Ghent* Additional information Secretariat of the Organizing Committee *c/o Holland Organiz*ing Centre 16 Lange Voorhout The Hague the Netherlands

20th International Congress conducted by the National Eye Research Foundation

will be an international contact lens congress November 16–24 1975 held in Las Vegas Nevada and in Acapulco Mexico The first section in Las Vegas will feature general sessions orthokeratology and photo keratoscopy section meetings exhibits and a *special one day amphitheater type workshop* featuring specialists in fitting patients with soft and hard lens bifocals orthokeratology procedures etc using different kinds of equipment with results monitored by special panels Information Frank Ciszek Executive Director 18 South Michigan Avenue Chicago Ill 60603 U S A

Vth Congress of the European Society of Ophthalmology

will be held at the Congress Centre Hamburg Federal Republic of Germany 5–9 April 1976

The European Society of Ophthalmology was founded in 1958 and has since convened in Athens Amsterdam Vienna and Budapest

The main theme of the Congress in 1976 will be a study of the circulation of the uvea retina and optic nerve (physiology and pathology) In addition about 120 free papers will be presented films will be shown and scientific technical and book exhibitions will be held

Congress Committee Professor H Sautter (President University Eye Clinic Hamburg) Dr H Rossmann (Secretary general University Eye Clinic Hamburg)

Further information Congress Secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague the Netherlands

The University Eye Clinic Rigshospitalet Copenhagen (Heads E Gregersen V Dreyer J Edmund S Kessing and H H Seedorff)

THE NONCONTACT TONOMETER

Clinical Evaluation on Normal and Diseased Eyes

BY

PER NELLEMANN SØRENSEN

The measurement of intraocular pressure with a noncontact tonometer was correlated to a Goldmann tonometer in 20 normal persons 20 glaucomatous patients and 8 patients suffering from corneal diseases
In normal persons the error in measurement for the noncontact tonometer was related to their skill in fixation, and in eye patients to height of pressure and corneal state
Acceptable correlation was found between noncontact tonometry and Goldmann applanation tonometry when the cornea was normal and the pressure below 35 mmHg (Goldmann) otherwise noncontact tonometry was only a guide and in the presence of corneal disease unreliable. Good fixation reduced the method error The standard deviation was 1 09 mmHg at poor fixation and 0 60 mmHg at good fixation
Repeated measurements on the same eye with noncontact tonometry did not alter the intraocular tension

Key words applanation tonometry – fixation – glaucoma – Goldmann tonometry – intraocular pressure – noncontact tonometry

In 1971 Grollman took out a patent for a noncontact tonometer and in 1972 he published the principle of this means of measuring the intraocular pressure
The clinical use of this instrument was first described in 1974 by Forbes Pico & Grollman There is however disagreement about the measuring accuracy of the instrument on normotensive subjects (Stepanik 1974) as compared with the Goldmann applanation tonometer to which the noncontact tonometer is calibrated The noncontact tonometer (NCT) was investigated

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on normotensives with special reference to fixation skill since this is an important factor and has never been investigated

Furthermore the measuring accuracy of the *instrument was studied* on hypertensive (> 24 mmHg) and normotensive eyes with pathological corneae because there are reasons to suppose that in using the NCT the measuring error will affect these eyes particularly The hypertensive eyes investigated included both eyes with clear oedematous or scarred corneae

The construction and function of the instrument is described by Grollman (1972) and Forbes et al (1974) and is summarized in the following

With linear increasing power an air current applanates a central area of 3 6 mm in diameter A pencil of rays is directed towards the central area oblique to the air current On the other side of the air current a photoelectric cell records the reflected light With increasing air current power the corneal surface changes its form from a steeper to a flatter convex mirror thus directing more light to the photoelectric cell The time for reaching maximum light intensity indicates the time for applanation. This interval is thus a measure for the intraocular pressure and the time is measured by an oscillator clock released at the same time as the air current. The time interval is transformed from the clock to a recorder (digital display) to millimetre mercury the tonometer being calibrated to the Goldmann tonometer

The time interval for normotensive eyes averages 2 msec The instrument is adjusted by a system similar to that of the radioscope a red dot target must be focused and placed in the centre of a white circle A mirror system ensures that the examinee also looks towards the red dot target which in relation to the examinee is imaged in an optically infinite distance

Another system (Secundary Automatic Alignment Verification System) ensures that the air current and the pencil of rays hit the optical centre of the cornea The system consists of infrared light which is sent out in the same direction as the air current is perpendicularly reflected and received in a detector If the red dot target falls outside the white circle the AAVS ensures that the tonometer button cannot be released

Materials and Methods

In experiment I the intraocular pressure (IOP) was measured on 20 normal eyes only one eye in each person With the noncontact tonometer 10 measure*ments on the same eye were made successively* The noncontact applanation was made with American Optical s instrument HS 1258 At the first measurement the time from the discovery of the red focusing dot target to the *fixation* of the dot target within the white circle is read on a stop watch this time interval being taken as expression of the fixation *skill* The IOP was measu ed every 1/ min The subjects were seated comfortably

A few minutes after the NCT recording one single Goldmann applanation measurement was made

The Goldmann applanation tonometry was made with Haag Streit s model mounted on Haag Streit slit lamp model 500 on which the reading was covered by paper so that the adjustment could not be read till after the procedure Novesin® (oxibuprocainum NFN) local anaesthetic with diluted fluorescein (0 125 %) was given and the adjustment was made exactly between the cardiac cycles

In experiment II the measurements were made on hypertensive eyes with clear cloudy or scarred corneae and normotensive eyes with pathological corneae one eye in each person The IOP was measured with the NCT and Goldmann applanation tonometry as in experiment I omitting the timing Measurements were made on

a) 14 eyes which presumably had increased IOP due to simplex glaucoma and with clear cornea judged by slit lamp examination Visual acuity was better than 6/9

b) Six patients with acute glaucoma and oedematous cornea

c) Eight patients with normal pressure and irregular cornea due to keratitis keratoconus or leucoma

The automatic verification system was disconnected during b) and c)

Table I

One single Goldmann applanation compared with noncontact applanation in 20 normal eyes (one eye / subject) 10 succesive measurements are performed every half minute The subjects are divided into two groups according to ability of fixation e g short and long adjustment time

	Goldmann applanation	Noncontact applanation		
	Mean reading (max./min)	Mean of first reading (max./min)	Mean of lowest reading (max./min)	Mean of mean readings in each subject ± standard deviation
Adjustment time > 3 sec n = 11	15 09 mmHg (10 / 20)	15 78 mmHg (11 / 20)	14 45 mmHg (10 / 20)	15 62 mmHg ± 1 09 f = 99
Adjustment time < 3 sec n = 9	15 22 (10 / 20)	15 67 (10 / 20)	14 89 (10 / 20)	15 66 ± 0 60 f = 81

Table II

One single Goldmann applanation compared with noncontact applanation in patients with elevated IOP and pathological cornea The patients with elevated IOP are divided into classes according to tension and present corneal oedema
10 successive noncontact measurements are performed every half minute on one eye in each

	Goldmann applanation	Noncontact applanation		
	Mean reading (max./min.)	Mean of first reading (max./min.)	Mean of lowest reading (max./min.)	Mean of mean readings in each patient ± s d
Elevated IOP $35 \geq X > 24$ n = 8 Clear cornea	30 88 mmHg (26 / 35)	3[illegible] 50 mmHg (43 / 55)	32 00 mmHg ([illegible]4 / 38)	33 08 mmHg ± 1 06 f = [illegible]
Elevated IOP $X > 35$ n = 6 Clear cornea	41 00 (36 / 47)	51 00 (43 / 55)	48 83 (44 / 50)	51 43 ± 2 0[illegible] f = 54
Elevated IOP n = 6 Corneal oedema	[illegible]3 00 (42 / 66)	72 00 (61 / 90	66 00 (61 / [illegible]4)	[illegible]9.90 ± 5 44 f = 54
Normal IOP n = 8 Pathological cornea	19 00 (17 / 25)	21 38 (18 / 28)	16 50 (13 / [illegible]0)	[illegible]0 66 ± 5 55 f = 7[illegible]

Results

Normal eyes

Table I demonstrates that 10 repeated noncontact applanation measurements with half minute intervals do not indicate major deviation between the measurements The adjustment time of the instrument was taken as a measure of the fixation skill and the normal eyes were arbitrarily subgrouped into two groups according to whether the adjustment was relatively quick (< 3 sec) or time consuming (> 3 sec) However reservations must be made regarding the division of the population into good or bad fixators

It is worth noticing that if the adjustment of the instrument is more time consuming (> 3 sec) e g if the fixation is poor standard deviation will in

crease. Bartlett's test showed variance homogeneity and a common standard deviation could be calculated.

The standard deviation is thus 0.60 mmHg at good fixation (< 3 sec) and 1.09 mmHg at poor fixation (> 3 sec). All error is attributed to the NCT measurement. If the corresponding estimations of the variances are F tested disparity is found ($P = 0.05$ two tailed) and consequently it may be assumed that the two groups (good or poor fixation) cannot be regarded as belonging to the same population.

Table III demonstrates good correlation between Goldmann tonometry and the NCT in the first, the lowest, and the mean of 10 readings. However it is more relevant that the corresponding measurements with Goldmann

Table III

Correlation between Goldmann and noncontact (NCT) applanation

	Goldmann/First NCT reading			Goldmann/Lowest NCT reading			Goldmann/Mean of 10 readings		
	r	b	$s_{\bar{t}}$	r	b	$s_{\bar{t}}$	r	b	$s_{\bar{t}}$
Normal IOP n = 9	0.98	1.01	1.06	0.98	0.90	0.80	0.98	0.96	0.89
Adjustment time < 3 sec	0.001	0.001		0.001	0.001		0.001	0.001	
Normal IOP n = 11	0.77	0.56	[illegible]	0.88	0.62	1.54	0.91	0.63	1.32
Adjustment time < 3 sec	0.001	0.01		0.001	0.001		0.001	0.001	
Elevated IOP n = 8	0.92	[illegible]	1.96	0.96	1.40	1.42	0.94	1.37	1.91
Clear cornea									
35 ≥ X > 24 mmHg	0.001	0.005		0.001	0.001		0.001	0.001	
Elevated IOP n = 6	0.53	0.90	[illegible]	0.56	0.42	3.15	0.53	0.55	[illegible]
Clear cornea									
X > 35 mmHg	–	–		–	–		–	–	
Elevated IOP n = 6	0.53	0.58	9.93	0.49	0.23	5.35	[illegible]	0.58	[illegible]
Corneal oedema	–	–		–	–		–	0.05	
Normal IOP n = 8	0.37	0.94	[illegible]	0.60	0.40	[illegible]	0.67	0.56	2.55
Pathologic cornea	–	–		–	–		–	–	

r = coefficient of correlation. b represents $b_{y,x}$ = regression coefficient.
$s_{\bar{t}}$ = standard deviation of mean NCT.
The significance limits for difference from zero for r and b are shown. (–) denotes that r or b are not different from zero for $P = 0.05$.

Table IV

Correlation between Goldmann and noncontact (NCT) applanation s^D = standard deviation between pairs of NCT and Goldmann measurements m^D = the mean difference between pairs of NCT and Goldmann measurements

	Goldmann/First NCT reading		Goldmann/Lowest NCT reading		Goldmann/Mean of 10 readings	
	s^D	m^D	s^D	m^D	s^D	m^D
Normal IOP n = 9 Adjustment time < 3 sec	1 01	0 44	0 8[illegible]	–0 31	0 [illegible]5	0 [illegible]1
Normal IOP n = 11 Adjustment time < 3 sec	2 09	0 64	2 29	0 37	1 95	0 4[illegible]
Elevated IOP n = 8 Clear cornea 35 ≥ \ > 24 mmHg	3 10	3 20	4 25	4 00	2 85	[illegible] 38
Elevated IOP n = 6 Clear cornea \ > 35 mmHg	4 20	10 0	5 31	5 07	3 78	10 40
Elevated IOP n = 6 Corneal oedema	10 0	2[illegible] 10	7 60	13 00	7 56	0 90
Normal IOP n = 8 *Pathologic cornea*	4 74	4 00	2 41	–0 50	3 51	3 45

tonometry and the NCT do not show great difference between the two types of measurements regarding the first the smallest and the average of 10 readings (Table IV) Thus the standard deviation of the difference was 1 01 mmHg at good fixation and 2 09 at poor fixation

Also with the Wilcoxon test for paired differences the H_0 hypothesis (hypothesis of no differences) is accepted for $P > 0\ 10$ (two tailed)

Hypertensive eyes clear or oedematous corneae and normotensive eyes with pathological corneae

Table II demonstrates that the standard deviation is small (1 06 mmHg) if the cornea is clear and the tension below 35 mmHg If on the other hand th cornea is oedematous and the tension more than 35 mmHg the standard deviation will be considerable and completely unacceptable in oedematous

and pathological cornea (Bartlett s test showed variance homogeneity for the 10 readings in the subgroups permitting the calculation of a common standard deviation)

Table III demonstrates that if the cornea is clear and the tension less than 35 mmHg an acceptable correlation between Goldmann and noncontact tono metry is found

Furthermore the standard deviation of the differences between pairs of Goldmann and NCT measurements was low 3 10 mmHg at the first reading *With the Wilcoxon test there is no significant difference between the two* types of measurements The H_0 hypothesis will be accepted for $P > 0\ 10$ (two tailed) whether the first lowest or average of the 10 readings is used

If the tension is higher than 35 mmHg or the cornea is irregular no correla tion between Goldmann and noncontact applanation is found whether the first lowest or the average of the 10 readings is used The standard deviation of the paired differences supports this statement (Table IV) The Wilcoxon test also shows difference in the two types of measuring ($P > 0\ 01$ two tailed)

Repeated noncontact applanation

Table V indicates that 10 NCT measurements every half minute leave the IOP unchanged if IOP is below 35 mmHg and if the cornea is normal (Wilcoxon test H_0 accepted for $P > 0\ 10$ two tailed)

Table V

Repeated noncontact applanation every half minute for 5 min in normal subjects and glaucoma patients with intraocular pressure below 35 mmHg (Goldmann) The mean of the first and tenth measurement is shown s d is the standard deviation.

	1st measured tension ± s d	10th measured tension ± s d
Normal eyes		
Adjustment time > 3 sec n = 11	15 73 ± 2 13 mmHg	15 83 ± 1 95 mmHg
Adjustment time > 3 sec n = 9	15 67 ± 2 90	15 54 ± 1.98
Flevated IOP 35 > \ > 21 Clear cornea n = 8 (one eye/subject)	32 50 ± 4 78	33 12 ± 5 93

Discussion

The investigation has shown that the standard deviation s^D of differences between pairs of NCT and Goldmann measurements was lower than 3 10 mmHg provided the intraocular tension is below 35 mmHg and the cornea normal

Also but less relevant a high correlation coefficient ($r > 0\ 9$) was found between the paired NCT and Goldmann measurements under the same circumstances

Forbes et al (1974) found a similar correlation coefficient ($r = 0\ 9$) and a similar standard deviation between pairs of NCT and Goldmann measurements ($s^D = 2\ 86$ mmHg) among 570 eyes and their results were supported by Grollman (1972) who among 168 normal eyes found $r = 0\ 83$ and $s^D = 2\ 0$ mmHg The simple comparative study of Ducrey et al (1975) involving 90 eyes with mixed tensions but no stratification also shows satisfactory agreement between the NCT and Goldmann measurements

Forbes et al who measured on hypertensive and normal eyes made no stratification but a considerable discrepancy seemed to exist between the Goldmann and the NCT applanation with tensions above 40 mmHg

In comparative studies on normal eyes the average of several NCT measurements might be more accurate than the first measurement (Stepanik 1974) Furthermore Forbes et al (1974) stress that the lowest reading is the most accurate when fixation is poor or impossible These statements may be true but they have not been definitely substantiated in this study

The present investigation proves that the method error is reduced by good adjustment and good fixation In amblyopic patients who cannot fixate exactly the method error may be intensified as was assumed but not investigated by Forbes et al (1974) The method error in normal eyes was found to be considerably less (s d $= 0\ 60 - 1\ 09$ mmHg) than suggested by Stepanik (1974) Stepanik measured the intraocular pressure in both eyes of 10 normal persons by 10 fast NCT measurements followed by 10 Goldmann applanations He found the standard deviation to be 2 37 mmHg by the NCT and 0 60 mmHg by Goldmann applanation

With repeated applanation the IOP decreases 3–4 mmHg in average during 5 min and the drop in tension is described as fairly exponential (Moses 1961 Krakau & Wilke 1974) This decrease exhibits a considerable individual variation With series of Goldmann applanations a systematic error will be found which exceeds the method error found by Stepanik

The NCT instrument does not provoke a decrease of tension by measuring every half min for 5 min as stated by Forbes et al and verified in the present work (The vibration tonometer has the same characteristic (Krakau 1970))

In practice the NCT measurement has proved to be favourable in certain respects This applies particularly to children and eye squeezers where the instillation of eye drops and Goldmann applanation tonometry or Schiotz tonometry is unreliable or entirely impossible

If the patient has a corneal disease the present investigation confirms the suspicion that the NCT measurement is not reliable. However Goldmann applanation tonometry would also be unsatisfactory in these circumstances With an IOP exceeding 35 mmHg the NCT instrument could still be used as a guide to indicate whether or not the eye is normotensive

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ENDOPHTHALMITIS FOLLOWING CATARACT EXTRACTION

A study of 24 cases in 4 498 operations

BY

J A FAHMY

The incidence of postoperative endophthalmitis in a series of 4 498 consecutive cataract operations performed in a period of 10 years was examined and found to equal 0.533 %.

Paracentesis of the anterior chamber was done in two cases and revealed *Staphylococcus albus* to be the causative organism in both instances Conjunctival cultures were recovered in a further 17 cases and showed *S albus* in pure culture in eight cases and in combination with *Pseudomonas aeruginosa* and *Proteous morgani* in two cases *Streptococcus haemolyticus* was isolated in pure culture in one case while the cultures from six other patients were negative

The role of such factors as age diabetes mellitus chronic bronchitis weak ocular tissue and persurgical complications which predisposed to postoperative infections was examined and found to have no significance on the occurrence of endophthalmitis in the present study

The visual end results were assessed and showed a better prognosis than generally expected Of 24 cases five achieved good visual acuity (6/6–6/12) 13 had a useful vision (6/18–6/60) while five remained actually blind (< 6/60) In only one case was the eye enucleated

Methods of treatment and prophylaxis are described and discussed A total suppression or elimination of all regional bacteria at the time of surgery seems to be the logical goal

Key words endophthalmitis – postoperative infection – eye surgery – cataract extraction – bacteria – antibiotics – steroids

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During the course of 1 year while studying the bacterial flora in patients under going cataract surgery (Fahmy et al 1975 b c d e) the author witnessed three cases of postoperative endophthalmitis occurring in a series of 499 patients this was equal to an infection rate of 0 6 % While preparing the manuscripts a further six cases occurred within a period of 7 months

From the literature (Table I) it was seen that 0.5 % seems to be the average rate of postoperative endophthalmitis occurring at the present time However the above mentioned relatively frequent occurrence of postoperative endophthal mitis within such a short time suggested a rising incidence probably due to a deficiency in the aseptic technique of the department

The present study was undertaken for the following reasons to assess the overall rate of postoperative endophthalmitis occurring in the last 10 years in order to see whether the incidence was actually rising to analyse the cases of endophthalmitis in regard to the nature of the offending microorganisms rela tionship to factors predisposing to postoperative infections methods of treatment and visual outcome and finally to discuss methods of prevention on the basis of further studies on the bacterial flora in relation to cataract extraction (Fahmy et al 1975 b c d e)

Material and Methods

The material comprises the records of 4 498 consecutive cataract operations performed in a 10 year period from 1 4 1964 to 31.3 1974 at the Department of Ophthalmology Kommunehospitalet Copenhagen

The records were studied retrospectively with however the added data of 499 patients studied prospectively from 17 8 1972 to 16 8 1973 (Fahmy et al 1975b)

Prophylaxis and postoperative care Approximately 18–20 hours before surgery the lashes were clipped scopolamine eyedrops and a single dose of oxytetracycline poly myxin B ointment were instilled into the conjunctival cul de sac and the eye covered with a sterile pad and a shield. On the day of surgery mydriatics (10 % phenylephrine and 1 % cyclopentolate) but no antibiotics were administered In the theatre, the operative cutaneous field was washed with sterile soap and saline, and the area further disinfected with 5 % iodine alcoholic solution, except in cases with iodine allergy when alcohol alone was used At the conclusion of surgery a small amount of pilocarpine powder as well as chloramphenicol ointment were applied On the first postoperative day the area was washed with sterile saline solution and mydriatics (1 % atropine and 10 % phenylephrine) were then instilled into the eye Usually no antibiotics were administered before the fourth postoperative day and then mostly in combination with steroids as routine procedure

Postoperative endophthalmitis The diagnosis was based mainly upon the *clinical* manifestations hypopyon and extreme redness and chemosis of the conjunctiva, with/

Table I

Incidence of postoperative endophthalmitis as reported in the literature

Author	Period	Operations	Endophthalmitis	Rate %	Prophylaxis
Lindner (1914)	1907–1910	1943	28	1.441	Mercury bichloride
Ramsay (1921)	*1898–1921*	*2146*	*68*	*3.168*	Not stated – surgery only when conjunctiva was sterile
Parker (1927)	1905–1920	1421	10	0.703	Not stated
Edmund (1927)	1910–1925	1089	18	1.652	Mercury prec. ointment
Davenport (1928)	1919–1925	2368	29	1.224	Not stated
Berens & Bogart (1938)	1936–1937	1004	1	0.099	Silver nitrate 1%
Guyton & Woods (1943)	1926–1944	1144	12	1.048	Argyrol 10% boric acid *sulphonamides*
Dunnington & Locatcher Khorazo (1945)	1938–1945	2508	11	0.438	Protein silver 25% – cultures prior to surgery
Hughes & Owens (1947)	1925–1947	3286	23	0.699	Mainly none – penicillin in the last 2 years
Dellaporta et al (1948)	1938–1948	1725	21	1.217	Not stated
Liehn & Schlagenhauff (1952)	1948–1951	1000	2	0.200	Penicillin topically
Callahan (1953)	*1947–1952*	*1653*	*5*	*0.302*	Antibiotics in cases with potential pathogens
Cason & Winkler (1954)	1947–1952	1653	11	0.665	Antibiotics in cases with *potential pathogens*
Locatcher Khorazo & Gutierrez (1956)	1945–1955	7662	6	0.078	Cultures prior to surgery*
Pearlman (1956)	1948–1955	6201	13	0.209	Penicillin – streptomycin
Neveu & Elliot (1959)	1954–1958	1047	7	0.668	Mainly none – antibiotics in last part of study
Burns (1959)	1956–1958	2695	5	0.185	Mainly none – antibiotics in some cases
Allen & Mangiaracine (1964)	1950–1964	20000	22	0.110	Antibiotics topically
Freeman & Gray (1967)	1953–1964	8277	40	0.483	Not stated
Whiston (1967)	1955–1964	1754	10	*0.570*	*Antibiotics – topically*
Cassady (1967)	not stated	1212	0	0.000	Antibiotics subconj at conclusion of surgery
Locatcher Khorazo & Gutierrez (1972)	1955–1968	15278	19	0.124	Cultures prior to surgery*
Christy & Lall (1973)	*1957–1972*	*77093*	*382*	0.495	Antibiotics topically
Allen & Mangiaracine (1971)	1964–1972	16000	9	0.056	Antibiotics topically
Present study	*1964–1974*	*4498*	*24*	*0.533*	Antibiotics topically

* Antibiotics administered preoperatively based on bacteriological findings

without haziness of the cornea, swelling and oedema of the lids When endophthalmitis had been diagnosed treatment was started immediately after the recovery of conjunctival cultures Preoperative cultures were made only in the minor prospective group of 499 cases

Statistical methods Chi square significance test with Yates correction as the calculation was performed on 2 × 2 table

Results

Incidence

As seen from Table II during the 10 year period 24 cases of endophthalmitis occurred in 4 498 operations which is equal to an infection rate of 0 533 %. The incidence of endophthalmitis has varied in the study from none in the first year to 1 079 % in the last year

In order to examine whether the infection rate in the last years was actually increasing the incidence of endophthalmitis occurring in the last 2 years was compared with that of the first 8 years of the study Of 1 124 operations performed between 1 4 1972 – 31 3 1974 10 cases developed endophthalmitis (0 889 %) while in the period 1 4 1964 – 31 3 1972 14 infections occurred in 3 374 operations (0 414 %) The difference as tested by χ test was not significant (χ = 2 597 f = 1 $P > 0$ 1)

Table II

Incidence of postoperative endophthalmitis in a period of 10 years

	Period	Operations	Endophthalmitis	Rate %
1	1 4 1964 – 31 3 1965	329	0	0 000
2	1 4 1965 – 31.3 1966	340	2	0.588
3	1 4 1966 – 31.3 1967	306	1	0.368
4	1 4 1967 – 31 3 1968	337	2	0.518
5	1 4 1968 – 31.3 1969	464	3	0 647
6	1 4 1969 – 31.3 1970	498	3	0 60[illegible]
7	1 4 1970 – 31.3 1971	544	2	0.367
8	1 4 1971 – 31.3 1972	506	1	0 198
9	1 4 1972 – 31.3 1973	568	4	0 704
10	1 4 1973 – 31.3 1974	556	6	1 079
Total	1 4 1964 – 31.3 1974	4498	24	0.533

Table IV
Bacteriological data in three cases of postoperative endophthalmitis

Code	Bacterial culture: Origin	Time of sampling	Results: Case 16	Case 17	Case 18
A	Conjunctiva	On admission	*S albus* 73 col corynebacteria 150 col	*S albus* 150 col	*P morgani* > 300 col
B	Nose	On admission	*S albus* corynebacteria *E coli*	*S albus* corynebacteria	corynebacteria *E coli*
C	Skin of face	On admission	*S albus* *B anitratum*	*S albus* corynebacteria	*S albus*
D	Air ward	On admission	None*	None*	None*
E	Conjunctiva	Begin surgery	*S albus* 27 col	*S albus* 1 col	*S albus*†
F	Air theatre	During surgery	*B anitratum* 3 col	*B anitratum* 3 col	None
G	Surgeon ungloved finger samples	End surgery	*S albus*	*S albus*	*S albus*
H	Assistant ungloved finger samples	End surgery	*S albus*	*S albus*	*S albus*
I	Wound site	End surgery	*S albus*†	*S albus*†	*S albus*†
J	Conjunctiva	4th postop day	No growth**	*S albus* 30 col	*S albus* > 300 col *P morgani* 15 col
K	Nose	4th postop day	*S albus* corynebacteria *E coli*	*S albus* corynebacteria	corynebacteria *E coli* *P morgani*
L	Air ward	4th postop day	*B anitratum* 3 col	None*	None*
M	Conjunctiva	7th postop day	*S albus* 5 col	*S albus*†	No growth

* With particular reference to *S aureus* gram negative bacilli and streptococci
** Culture obtained after the administration of antibiotics
† Growth in serum bouillon but not on agar

Treatment and visual outcome

In the first years of the study (eight cases) chloramphenicol was administered systemically in a dose of 3 g daily as well as neomycin (20 mg) subconjunctivally and oxytetracycline polymyxin B topically In recent years (16 cases) ampicillin (1 g daily) has been substituted for chloramphenicol systemically gentamycin (20 mg daily) for neomycin subconjunctivally and topicin (bacitracin and neomycin) for oxytetracycline polymyxin B topically Prednisone was administered in 20 cases systemically with a starting dose of 60 mg daily

As to the visual results of the above mentioned treatment five patients achieved good visual acuity (6/6–6/12) 13 had useful vision (6/18–6/60) while five remained actually blind (< 6/60) In only one case was the eye enucleated (Tables V VI)

Discussion

According to Graefe (1878) in preantiseptic days postoperative endophthalmitis followed cataract extraction in approximately 10 % of the cases After the introduction of the Lister routine in ophthalmic surgery this rate was reduced to 2–3 % (Graefe 1878)

In the following years after the discovery of microorganisms as causative agents of postoperative endophthalmitis and by improving the methods of their destruction the infection rate could be reduced to an average of 1.5 % (Lindner 1914 Ramsay 1921 Parker 1927 Edmund 1927 Davenport 1928 Slocum 1933 Berens & Bogart 1938 Guyton & Woods 1943 Dunnington & Locatcher Khorazo 1945)

With the discovery of antibiotics the popularisation of aseptic techniques and due to the advances in surgical procedures the infection rate could be reduced to 0.5 % which is the average rate in most series published in recent years (Dellaporta et al 1948 Liehn & Schlagenhauff 1952 Callahan 1953 Cason & Winkler 1954 Pearlman 1956 Neveu & Elliot 1959 Freeman & Gray 1967 Whiston 1967 Christy & Lall 1973)

The incidence in the present study with 24 infections in 4 498 operations (0 533 %) is in keeping with that figure but still much higher than the 0 056 and 0 124 % recently reported by Allen & Mangiaracine (1974) and Locatcher Khorazo & Gutierrez (1972) respectively

While in the preantibiotic era pneumostreptococci were considered to be the major offenders in postoperative infections (Elsching & Ulbrich 1909 Gradle 1910 Imre 1911 Lowenstein 1911 v Liebermann & Lengyel 1911 Matafune & Albanese 1912 Lindner 1914 Kraupa 1914 Betti 1921 Ramsay 1921 Stanka

Table V
Methods of eatment and visual outcome in 24 cases of postoperative endophthalmitis

Code	Treatment									Visual outcome	Length of observation (months)
	Antibiotics								Steroids		
	Chlor amphe nicol	Ampi cillin	Genta mycin	*Topi cin	Framy cetin	Oxy tetr po lym B	Neo mycin	Sulpho namides			
01	C				A	A	B		A	6/6	51
02	C				A		B		A	6/24	2
03	C				A	A	B	A	A	6/24	2
04	C					A	B		C	6/24	22
05	C					A	B	A		excentr	
06	C		B			A		A	C	+L	8
07	C		B	A		A			C	6/24	38
08	C		B	A					C	6/24	3
09		C			A				C + A	1/18	3
10		C	B		A				C + A	6/36	3
11		C	B	A	A				C + A	6/24	2
12		C	B	A	A				C + A	1/24	3
13		C	B	A	A				C + A	6/9	3
14		C	B	A					C	6/9	2
15		C	B	A					C	2/18	5
16		C	B	A					C	+1	4
17		C	B	A					C	6/18	10
18		C	B	A					C	6/12	2
19		C	B	A					C	6/24	
20		C	B	A					C	6/18	2
21		C	B	A					C	6/36	2
22		C	B	A					C	6/60	1
23		C	B	A					C	6/9	2
24		C	B	A					C	[illegible]	[illegible]

Table VI

Visual end results following postoperative endophthalmitis as reported in the literature

	No of cases	6/6	6/9	6/12	6/18	6/24	6/36	6/60	< 6/60	+L	Enucleated	Not stated
Ramsay (1931)	68										46	22
Cuyton & Woods (1943)	12	2			2				1	7		
Dunnington & Locatcher Khorazo (1945)	11									5	6	
Hughes & Owens (1947)	23	1	2						3	9	7	1
Foster (1949)	7		2	1							5	
Dellaporta et al (1949)	21	2		4	2		1			12		
Callahan (1953)	5									2	3	
Cason & Winkler (1954)	11									2	9	
Custodis (1958)	12	6	2		2				1	1		
Pico (1958)	5		2							3		
Neveu & Elliot (1959)	7						1			5	1	
Allen & Mangiaracine (1964)	22		2	1					1	8	10	
Aronstam (1964)	8	1	1			1		1		3		
Lincus et al (196)	5	1	2	1								1
Whiston (1967)	10	1	4	2					1	–		
Allansmith et al (1970)	14	1	3			1			3		1	5
Allen & Mangiaracine (1974)	9		2						2	3	2	
Present study (197)	21	1	3	1	2	3	2	1	3	2	1	

1924 Wissmann 1924 McKee 1924 Lundsgaard 1925 Elsching 1926 Kuffler & Schneider 1926) *S aureus* (coagulase positive) as well as gram negative bacilli seem to be the main causative agents at the present time (Dunnington & Locatcher Khorazo 1945 Callahan 1953 Cason & Winkler 1954 Locatcher Khorazo & Gutierrez 1956 1972 Pearlman 1956 Burns 1959 Allen & Mangiaracine 1964 1974 Whiston 1967 Kolker et al 1967 Allansmith et al 1969)

Elsewhere (Fahmy et al 1975d) it has been demonstrated that conjunctival cultures obtained on the fourth postoperative day could not be relied on to reflect the actual flora present at the time of surgery i e the time when infection was most likely to occur It was concluded that anterior chamber paracentesis was a better way of establishing the etiology of postoperative endophthalmitis

In the present study anterior chamber paracentesis was performed only twice in both cases *S albus* was cultured This may confirm the experience of some other authors (Valenton et al 1973 Allen & Margiaracine 1974 Forster 1975) that *S albus* hitherto considered as apathogenic may be the causative agent of postoperative endophthalmitis

Several authors (Guyton & Woods 1943 Dellaporta et al 1948 Locatcher Khorazo & Gutierrez 1956 Burns 1959 Allen & Mangiaracine 1964 Kolker et al 1967 Christy & Lall 1973) have noted that the rate of postoperative infection in extracapsular extraction was higher than in intracapsular extraction Others (Jaffe 1972 Christy & Lall 1973) have reported that vitreous loss was another disposing factor for endophthalmitis It is likewise stated (Burns 1959 Allen & Mangiaracine 1964 Kolker et al 1967 Christy & Lall 1973) that errors of technique multiple operations on the same eye prolonged operating time hospitalisation in a ward rather than in private accommodation weak ocular tissue severe metabolic disturbances such as diabetes and advanced age were factors leading to an increased incidence of infections

According to the present results none of these factors seemed to play any role in the development of endophthalmitis as the incidence of unintended extracapsular extraction vitreous loss diabetes as well as the average age of those patients with endophthalmitis was largely the same as that found in a sample of 499 patients examined elsewhere (Fahmy et al b d) Furthermore it may be pointed out that all patients constituting the present material were operated and hospitalised under the same conditions In addition none of the infected cases had weak ocular tissue or were reoperated on the same eye

It has also been stated (Ramsay 1921 Dunnington & Locatcher Khorazo 1945 Hughes & Owens 1947 Cason & Winkler 1954 Neveu & Elliot 1959 Allansmith et al 1970) that the end result of endophthalmitis following cataract extraction was very poor In 1921 Ramsay reported the loss of 46 eyes out of 68 The

results of Allen & Mangiaracine (1964 1974) were not much better out of 31 cases encountered in a period of 21 years 12 were eviscerated 11 remained with ± light perception three had < 6/60 while only five patients had better than that The end results of the present series with the loss of only six eyes (exentration – < 6/60) out of 24 belong apart from the results of Custodis (1958) and Pincus et al (1965) to the best ever reported (Table VI)

This could be due to the methods of treatment employed Antibiotics and steroids were administrated in sufficient doses immediately after the clinical diagnosis was made without waiting for the bacteriological results At the present time *gentamycin* and *ampicillin* are mainly used The spectrum of both is effective against the great majority of bacteria known to cause endophthalmitis

The efficiency of systemic steroids added to antibiotics in the treatment of postoperative infections has been disputed by some authors (Freeman & Gray 1967) who found no difference in the final results between patients receiving steroids and those treated with antibiotics alone

However in view of the fact that one may be dealing with a severe aseptic uveitis the advantages of their use appear to overshadow the risks they may incur

Furthermore the administration of steroids reduces the massive inflammatory response of the eye which in itself is often as destructive as the infection (Leopold 1972)

Some other factors which might have positively influenced the results of the present study could be that the infections were caused by low virulent micro organisms or that some of the inflammatory reactions were virtually due to aseptic uveitis

A culture from the anterior chamber taken immediately after the onset of symptoms therefore seems to be a logical procedure not only to differentiate between septic and aseptic endophthalmitis but also to reveal the causative organisms and determine their sensitivity The method which is not new (Axenfeld 1907) seems to be gaining a renaissance (Allansmith et al 1970 Valenton et al 1973 Allen & Mangiaracine 1974 Forster 1975)

As to the origin of postoperative endophthalmitis diverse sources of *exogenous* infection are known to exist Bacteria can be introduced into the globe by contaminated irrigating solutions or medications instilled during or after surgery (McCulloch 1943 Rintelen 1951 Rauh 1953 Custodis 1958 Allen & Mangiaracine 1964) Cotton pledgets (Cason & Winkler 1954) a nurse s septic arm (Ramsay 1921) and the nose of a surgeon (Allen & Mangiaracine 1964) were some other probable sources However the origin and routes in the great majority of cases remain undetected

On the other hand several ophthalmic surgeons (Axenfeld 1907 Elsching & Ulbrich 1909 Gradle 1910 Imre 1911 Metafune & Albanese 1912 Lindner 1914 Kraupa 1914 Stanka 1924 Lundsgaard 1925 Locatcher Khorazo & Gutierrez 1956 1960 1967 Allen & Mangiaracine 1964 1974 Ulrich & Burton 1974) have believed in the so called "*self infection*" theory i e infections caused by bacteria pre existing preoperatively on the patient s own conjunctiva

Recently Fahmy et al (1975c) have examined the origin of *S aureus* recovered from the wound site at the termination of surgery Out of 19 isolated strains 17 had either the same bacteriophage type complex (14 cases) or were not typable (3 cases) as those recovered from the conjunctiva on admission One strain could be traced to the nose of a surgeon while the origin of another could not be found as it was not typable

These results though none of the patients developed endophthalmitis may in some degree confirm the probability of the above mentioned theory

Consequently in methods of prevention a total suppression or elimination of all bacteria at the time of surgery seems to be the logical goal The average ophthalmic surgeon is not aware of the inefficiency of the preoperative treatment of the patient Elsewhere (Fahmy et al 1975c) it was demonstrated that 99 % of the patients receiving a single dose of oxytetracycline polymyxin B the after noon before operation proved to harbour one or more kinds of bacteria at the time of surgery

Locatcher Khorazo & Gutierrez (1972) stated that 2–3 days were required to eliminate *S aureus* from the eye gram negative bacilli could persist for as long as 7 days Their preoperative antibiotic regimens were usually suited to the organisms recovered on culture and lasted until preoperative cultures became negative Allen & Mangiaracine (1974) applied a short but intensive preopera tive regimen starting the afternoon before surgery Chloramphenicol 0 4 % and polymyxin B sulphate 0 1 % in a mixed collyrium were administered topically each hour and at bedtime erythromycin ointment 0 5 % was applied to the eye

Both series were rewarded by having the lowest incidence of postoperative endophthalmitis ever reported (Table I)

There exist different opinions about the value of preoperative cultures as a prophylactic tool Several authors (Elsching & Ulbrich 1909 Imre 1911 v Liebermann & Lengyel 1911 Metafune & Albanese 1912 Kraupa 1914 McKee 1924 Lundsgaard 1925 Rodin 1945 Dunnington & Locatcher Khorazo 1945 Callahan 1953 Cason & Winkler 1954 Smith 1954 Chang 1957 Locatcher Khorazo & Seegal 1972) believed that in revealing potential pathogens prior to surgery postoperative endophthalmitis might be prevented Others (Lindner 1914 Cooper 1935 Hughes & Owens 1947 Burns 1959 Nolan 1967 Allansmith et al 1969

Allen & Mangiaracine 1974) disagreed partly because postoperative infections can still develop despite their absence preoperatively

The present study may confirm this latter experience In addition it was observed elsewhere (Fahmy et al 1975c) that potential pathogens can be re covered from the wound site at the termination of surgery without the develop ment of any postoperative infection

On the basis of the present results and according to the literature, an adequate preoperative treatment with effective antibiotics administrated topically seems to be one of the best measures for the prevention of postoperative endophthal mitis This is in agreement with the conclusions of other authors (Allen & Man giaracine 1964 1974)

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THE IMMEDIATE RESPONSE IN APPLANATION PRESSURE TO INTRAVENOUS ACETAZOLAMIDE IN PRIMARY GLAUCOMAS AND GLAUCOMA SUSPECTS

BY

OLE I NISSEN

The decays in the applanation pressures of both eyes after a reduction of the aqueous secretion by means of intravenous acetazolamide are studied with respect to shape and steepness by comparison with reference curves calculated from different values of the facility of aqueous outflow The pressure decays in three out of four eyes with narrow angle glaucoma exhibited breaks possibly resulting from a stepwise reopening of the chamber angle during the decompression Twenty patients with intra ocular hypertension and open angles could be divided into two groups according to the facility of aqueous outflow (in μl mmHg $^{-1}$ min $^{-1}$) estimated from approximately matching reference curves Nine patients with facilities above 0 15 had normal visual fields and nearly indentical pressure curves of the two eyes Out of 11 patients with facilities below 0 15 seven patients had glaucomatous visual field defects and excavated optic discs The pressure curves of eye pairs in this low facility group showed asymmetry and breaks were seen in approximately half of the curves These might be caused by changes in the outflow facility due to a decompression of collapsed outflow channels However the morphological basis for such an interpretation is not as well founded as in the case of narrow angle glaucoma

Key words acetazolamide – applanation tonometry – facility of aqueous outflow – glaucoma test – intraocular hypertension – intraocular pressure – normal eyes

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If the formation of aqueous humour is suddenly reduced by an intravenous injection of acetazolamide the intraocular pressure begins to fall steeply after a couple of minutes eventually it flattens out and reaches a steady level (Linner & Wistrand 1959) In the present studies the intraocular pressure of both eyes of the sitting patient are followed by alternating measurements with a Goldmann applanation tonometer and it seems that the shape and steepness of the acetazolamide induced deflation curves *obtained in this way relate various* parameters from which a picture of the state of the aqueous circulation appears (Nissen & Hoppe 1974) In the following a general description of how the pressure curves have been evaluated is combined with a *presentation of the results obtained so far in primary glaucomas and glaucoma* suspects

Material and Methods

The material consists of four patients with narrow angle glaucoma and 20 patients with open angles pressures above 22 mmHg (measured on several occasions) and in some of the cases glaucomatous optic discs and visual field defects (see Results) Generally the test was carried out on untreated eyes in principle as described by Nissen & Hoppe (1974) and was combined with a routine examination including visual acuity gonioscopy ophthalmoscopy slit lamp examination and examination of the visual fields on the tangent screen

Summary of the method

The intraocular pressures of both eyes are measured alternately with a Goldmann applanation tonometer and registered together with time Fluress® (0 4 % oxibuprocain chloride and 0 25 % fluorescein) is used for staining the prism surface is kept clean from dried up substance During the *control period* normally lasting 15–20 minutes, the readings stabilize on *initial pressure levels* Acetazolamide is administered intra venously (10 mg per kg body weight) and the measurements are resumed and continued during the pressure decays The pressures stabilize on *final pressure levels* The pressure decays are compared with reference curves calculated (by a summation procedure) on the assumptions of a step fall in the secretion of aqueous humour due to the acetazolamide a well defined relation between the volume and the pressure of the eye (Friedenwald s equation with an ocular rigidity of 0 0215) different values of the facility of aqueous outflow and constant episcleral vein pressure Goldmann &Schmidt (1965) and Davanger & Holter (1967) obtained a virtually identical pressure time relation by integration In many of the experiments an immediate graphic representation of the experimental curves was obtained by using an applanation tono-

meter modified thus The axis of the adjustment button of the tonometer is connected to a turn potentiometer supplied with a constant voltage and the variable pressure dependent output of the potentiometer is fed into a servowriter with an electric penlift (e g Servogor® S Goerz electro Ges m b H A 1101 Wien) Each time the correct interlocking of the fluorescent archs is attained a button is pressed and the pen makes a dot on the paper By activating a two way contact (right eye/left eye) a fixed voltage can be added to the input of the writer so that the two pressure curves are separated on the paper (by the upward displacement of one of them) A standard sheet (A 4) of transparent millimeter paper fits the electronic writer

Results

Narrow angle glaucomas

The results of the test in three out of the four patients examined are illustrated in Figs 1 2 and 3 The fourth patient (male 59 years) had an initial pressure level of 31 mmHg in the diseased eye an estimated facility of outflow of

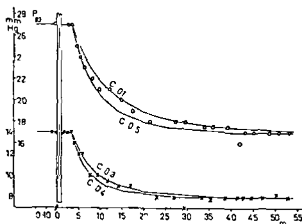


Fig 1

Man ? years Chronic angle closure (left) No medication The right eye was subjected to a successful fistulating operation 8 years before because of angle closure *Conclusions* The intraocular pressure versus time curves are monophasic and fit the reference curves well – no breaks are seen They show an abnormal aqueous circulation because of a) too high control pressure (left) b) too low facility of outflow (left) (c) unidentical curves – partly due to the operation) x right eye o left eye Vertical bar 10 mg acetazolamide per kg body weight i v

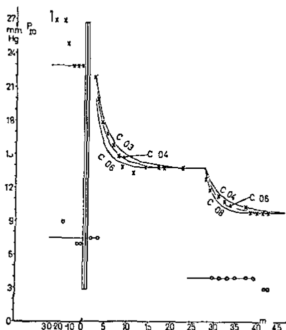


Fig 2

Woman 69 years Prodromal attack of angle closure (right) Two years before a filtering operation was made on the left eye because of angle closure The change after acetazolamide occurs in two phases The magnitude of the first outflow facility indicates together with an initial pressure close to normal that the drainage channels are near their full transport capacity At the start of the second fall one can imagine that probably due to the lessened compression of the iris root again t the trabeculae the iris folds are loosened in the remaining zone of contact and fall back like a spring resulting in a stepwise augmentation of the outflow facility Again there is a surplus of chamber fluid that has to drain through all available channels and the reference curves may be used to give a rough idea of the new and larger level of the outflow facility *Conclusions* The curves show an abnormal aqueous circulation because of a) a biphasic curve (right) regarded as an extreme example of a break b) too high control pressures (right) c) too low pressures (left) and d) unidentical curves – partly due to the operation For symbols see Fig 1

approximately 0 12 and a break in the curve resembling that of the left sided curve of Fig 8 A break is defined as a marked deviation in the course of the curve compared with the reference curves (see Figs) Thus in three out of four patients in this group the pressure curves showed one or more breaks

Open angle glaucomas and glaucoma suspects

In Fig 4 each vertical line is the response to acetazolamide in one untreated eye The abscissa (logarithmic scale) is the facility of outflow estimated from approximately matching reference curves and the ordinates the initial ($P_{10\ i}$) and the final ($P_{10\ f}$) intraocular pressure level For comparison the results of the acetazolamide test in 17 normal eyes (Nissen & Hoppe 1974) are plotted in the same graph A facility of outflow (C) of approximately 0 15 divides the material into two groups (A and B)

A) C > 0 15 This group includes 1) Nine pairs of hypertensive eyes from six women and three men with an average age of 58 years (range 45–75) Figs 5 and 6 (Fig 4 in Nissen & Hoppe 1974) The visual fields and the optic discs of these nine patients were without glaucomatous defects The average of the facilities of outflow was 0 33 Characteristic of their tests is (as in normotensive eyes) a high degree of identity between the two curves of a pair Thus the averages of the first pressures measured in a pair of eyes are

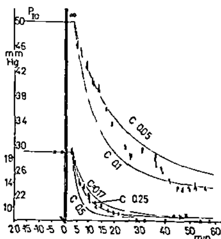


Fig 3

Woman 66 years Attack of angle closure (left) *Conclusions* The curves show an abnormal aqueous circulation because of a) unidentical curves b) too high control pressures (left) c) too low outflow facility (left) and d) breaks (left) – for definition of these see Results *Methodological comments* Most of the applanation marks are pieces of lines produced by the servowriter (see Methods) during protracted applanations This technique gives good precision but demands much concentration from both the patient and the examiner For symbols see Fig 1

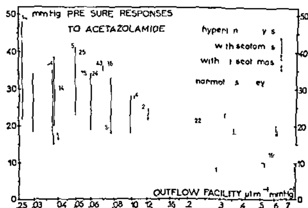


Fig 4

Intraocular pressure responses to intravenous acetazolamide in open angled untreated eyes Each vertical line is the response of one eye Abscissa logarithmic facility of aqueous outflow in μl min^{-1} mmHg^{-1} Ordinate applanation pressure in mmHg The responses of the normotensive and the hypertensive eyes with outflow facilities above 0 15 generally appear in pairs The responses of the remaining eyes are as a rule unidentical and therefore appear singly These responses are marked by the serial number of the patient for easier identification For details see text

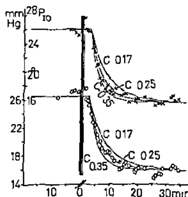


Fig 5

Woman 75 years Intraocular hypertension typical of the high outflow facility group ($C > 0$ 15) The visual fields were normal and the optic discs without excavation. The patient was retested again without eye medication Less frequent readings were used this time the curve parameters were well defined and unchanged *Conclusion* The only sign of abnormality is too high control pressures For symbols see Fig 1

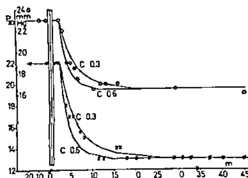


Fig 6

Woman 45 years Intraocular hypertension atypical case $C > 0.15$ The visual fields were normal the optic discs were centrally excavated and of good colour This result is shown because the curve of the left eye as the only one in the high outflow facility group includes an irregularity which might resemble a break – see Results The patient was retested under pilocarpine treatment (she was untreated the first time) The outflow facilities were unchanged the initial pressure levels 1–2 mmHg lower breaks could not be seen but the unusual large difference between the pressures on the final level remained (on pilocarpine 14/10 1/ mmHg left/right) *Conclusions* The test shows abnormal aqueous circulation because a) the control pressures are too high b) the curves are not completely identical c) a questionable break appears on the left side For symbols see Fig 1

24.6/24.5 mmHg (ranges 21–28/22 1/2–30 right/left) with an average difference between the eyes in a pair of 2.4 mmHg (range 1 1/ –4) The average initial pressure levels (i e where the control pressures have become stable) are 22.6/22.3 mmHg (ranges 20–26/19–27) with an average difference of only 0.8 mmHg (range 0–2 1/2) Two of the curves show irregularities in shape and one of these is comparable in magnitude to those of the low facility curves (see below and Fig 6 left) The test was repeated (again without treatment) in one patient (Fig 5) and gave the same response as the first time Four were retested during pilocarpine treatment giving a small fall in the control pressures and a questionable increase in the outflow facilities Included in the high outflow facility group were also 2) Four single normo or hypertensive eyes with glaucomatous fellow eyes and 3) Seven pairs of normotensive eyes (Fig 4)

B) $C < 0.15$ (average 0.07) This group includes seven pairs and four single eyes from three women and eight men with an average age of 62 years (range 50–69) In seven out of the 11 patients the visual fields of one or both eyes

were definitely pathological (residual central islands Bjerrum scotomas large nasal steps) and the discs were cupped The eyes were all hypertensive some of them to a large degree The averages of the first pressure readings are 34 5/32 0 mmHg and of the initial pressure levels 34 4/31 0 mmHg (in one eye the pressure increased by 4 mmHg in the control period in two eyes of two pairs it fell by 4 mmHg in the remaining eyes the changes were 0–2 mmHg up or down) The degree of asymmetry of the eyes is fairly large as the difference between the first pressures of a pair averages 9 9 mmHg (range 3–20) and between the initial pressure levels 10 9 mmHg (range 3½–20½) About half of the curves show breaks (defined above) and these were present both in curves from eyes with (cases 22 24 54) and without visual field defects (cases 5 22 24 53) and also in eyes with relatively low tensions (cases 22 24 53) Case 24 had the test made twice without treatment (Fig 4 and 11) Five patients were tested also with miotics (cases 16 24 25 53 54) the curves showed improvements in the circulatory parameters but they still indicated disease

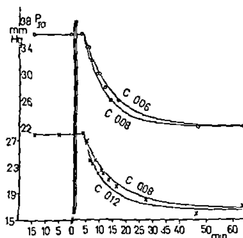


Fig 7

Case 43 man 60 years Intraocular hypertension C < 0 15 No eye treatment Visual fields without defects the optic discs without excavation Monophasic well fitting curves In the following months the hypertension showed a malignant course *Estimated* outflow facilities 0 1/0 07 (right/left) *Conclusions* The test shows abnormality of the aqueous circulation because a) the curves are unidentical b) the control pressures are *too high* c) the facilities of outflow are too low The patient belongs to the low outflow facility group see Fig 4 For symbols see Fig 1

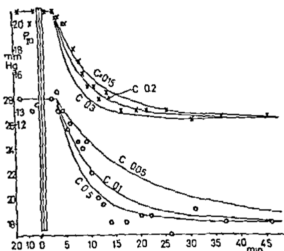


Fig 8

Case 22 man 65 years *Glaucoma* C < 0 15 (right) No eye treatment A nasal step was detected on the left side both discs were excavated nearly to the border and of light colour Breaks are seen at 6 min (right) and 9 min (left) Estimated outflow facilities 0.2/0 1 (right/left) *Conclusions* The test shows abnormality of the aqueous circulation because a) the curves are unidentical b) the control pressures are too high c) the estimated outflow facility of the left eye is too low d) breaks are present. For symbols see Fig 1

The usual side effects of acetazolamide (a cutaneous tingling or a metallic taste) were noticed by some of the patients but never caused any interruption of the test

Discussion

When evaluating the pressure versus time relations obtained in a pair of eyes with the present technique attention was paid to the following six parameters

1) The first pressure readings 2) the initial pressure levels 3) the final pressure levels 4) the facilities of aqueous outflow 5) possible breaks in the curves 6) the degree of similarity between the curves of the two eyes

1) The first applanation readings of the control period corre pond to those which in the everyday ophthalmological routine are often considered sufficient for an estimation of the pressure state During the control period the pressures gradually stabilized on levels which in most eyes were a few mmHg lower

than the first pressures On an average the control pressures fell 2 mmHg in intraocular hypertensive eyes belonging to the group with facilities of outflow above 0 15 and just like in the normal eyes the fall was absent in some and *reached 4–5 mmHg in other pairs* The major part of this fall is probably of reflex nature (Krakau & Wilke 1974) In the eyes with an outflow facility below 0 15 (and open angles) on an average no changes occurred in the control pressures

2) The standard of accuracy of applanation tonometry is regarded as high especially when used on a comparative basis on a pair of eyes or by consecutive measurements on the same eye For this reason the *initial pressure levels* (determined by the readings just before and after the acetazolamide injection) are well defined and are probably more apt to give an impression of the pressure status (including a comparison between the two eyes) than the first measurements (Leydhecker 1973 b) The initial pressure levels of the eyes in a pair were nearly identical in the hypertensive eyes of the high outflow facility group but differed more or less in the remaining hypertensive eyes (Fig 4)

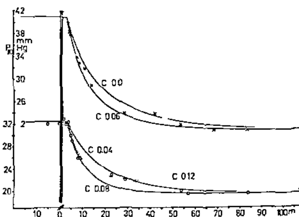


Fig 9

Case 5 woman 69 years *Glaucoma* C < 0 15 No eye treatment Cupped discs and severe defects in the visual field of the right eye *The first part of* the left sided pressure decay fits the dotted reference curve well but the experimental curve changes direction (breaks) 45 min after the injection and reaches a lower level than expected with a monophasic course The irregularity in the right sided curve is not accepted as a break Estimated outflow facilities 0 05/0 06 (right/left) *Conclusions* The test shows abnormality of the aqueous circulation because a) the curves are unidentical b) the control pressures are too high c) the outflow facilities are too low d) a break is present (left) For symbols see Fig 1

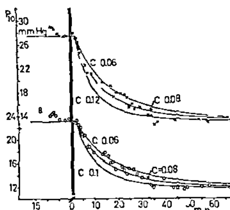


Fig 10

Case 53 woman 54 years Intraocular hypertension C < 0 15 No eye treatment The visual fields and the optic discs were normal The pressure of the right eye was always 4 to 5 mmHg higher than that of the left eye both responded well to pilocarpine drops The patient was calm relaxed and cooperative during the whole procedure The irregularities of these curves are similar to those seen in glaucomatous eyes with visual field defects They have been observed monolaterally as well Estimated outflow facilities 0 08 *Conclusions* The test shows abnormality of the aqueous circulation because a) The eyes are asymmetric with respect to pressure levels b) the control pressures are too high c) the outflow facilities are too low d) breaks are present in both curves For symbols see Fig 1

3) The initial pressure level ($P_{10\ init}$) minus the final pressure level ($P_{10\ t}$) gives the pressure reduction in mmHg produced by the acetazolamide If we knew the episcleral vein pressure P_V it would be possible to calculate the pressure reduction in per cent of the maximum reduction obtainable at complete secretory inhibition (for this purpose assuming that P_{10} equals P_V at zero secretion) as

$$100 \quad \frac{P_{10\ init} - P_{10\ t}}{P_{10\ init} - P_V} \ \%$$

Since no difference in episcleral vein pressure has been noted between normal and glaucomatous eyes (Duke Elder 1968) we assume that P_V equals 7 mmHg in all cases This is an acceptable value for sitting persons which furthermore harmonizes well with an average inhibitory effect of acetazolamide on the aqueous secretion of 50 % in a series of normal eyes (Nissen & Hoppe 1974) The efficiency of acetazolamide calculated in this way in the nine pairs of hypertensive eyes with outflow facilities above 0 15 become 49 % ± 7 (s d) In

35

the 11 pairs with facilities below 0 15 it is higher viz 58% ± 9 (s d) The difference is statistically significant both based on single eyes ($P < 0.01$) and on pairs ($P < 0.02$ Student s t test) and might be explained by a reopening of valve like outflow hindrances in the diseased drainage system (see point 5) (Evidently the difference in the efficiencies might also indicate that for some reason the secretory process in glaucomatous eyes is more susceptible to acetazolamide than in normal eyes) The phenomenon may be noticed in Fig 4 in the form of extraordinarily large pressure reductions in the group with low facilities of outflow (the reductions average 15 0 mmHg in this group compared to 7 6 mmHg in the high outflow facility group and 4 8 mmHg in the normotensive eyes)

4) The general steepness of the acetazolamide induced pressure decays gives together with a knowledge of the final pressure level an idea of the easiness by which the eve ball is able to eliminate superfluous fluid and reach a new *steady state with respect to pressure (and volume) A comparison with the* reference curves should allow a quantitative estimate of this easiness in terms of the facility of aqueous outflow From a theoretical point of view such a

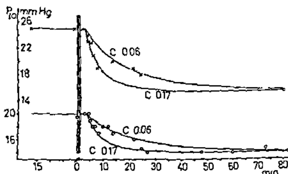


Fig 11

Case 24 *woman 66 years Glaucoma* C < 0 15 No eye treatment Open angles A large nasal step is found on the right side on the left the visual field is normal The *right optic disc is more excavated than the left but neither of them to the border* The curves are examples of a bad technique (unevenly distributed applanations) The test was repeated 18 days later again without eyedrops This time the initial pressur levels were 34/25 mmHg (right/left) there were breaks in the curves and the outflow facilities were low (0 06/0 19 see Fig 4) *Conclusions* The test shows abnormality of the aqueous circulation because a) the curves are unidentical (e pecially *with respect* to the control pressures) b) the control pressure is too high on the right side c) *the outflow facilities* are too low (but ill defined) d) questionable breaks are en in the curves For symbols see Fig 1

comparison is only correct if the facility of outflow stays constant during the pressure fall (i e when Poiseuille s law is valid Nissen & Hoppe 1974) Examples of this are probably the smooth and monophasic curves in Figs 1 and 7 From a practical point of view the comparison seems justified also in irregular or polyphasic curves (i e with breaks) where the facility of out flow may be changing since in addition to the apparently pathological out flow facilities reached through the reference curves such curves show other signs of disease (high pressures asymmetry) or originate in eyes with glau comatous scotomas and cupping of the optic discs Another point which tends to make the estimate of the facility of outflow inaccurate is that the reference curves are calculated on the basis of a single normal ocular rigidity (0 0215) in spite of the fact that it is well known that this parameter varies somewhat from eye to eye and in the same eye changes with the intraocular pressure and volume (Duke Elder 1968 Thorburn 1973) Nor has pseudofacility (Barany 1963 Kupfer 1973) been taken into account in the reference curves – these were calculated on the assumption that the rate of the (acetazolamide dominated) secretion of aqueous humour was constant during the pressure decay (Nissen & Hoppe 1974) In one case (Fig 2) a comparison with the reference curves in the usual way was impossible because the different phases of the decay were clearly separated in time In spite of these reservations it is evident that the estimated facilities of outflow give rise to a division of pairs of eyes with open angles into two groups one consisting of normal eyes and eyes with intraocular hypertension as the only sign of abnormality and another consisting of clearly glaucomatous eyes or eyes strongly suspected of disease in the aqueous circulation because of extra high intraocular pressures or asymmetry The limit between these groups is about $C = 0\ 15$ μl min^{-1} mmHg^{-1} a value that coincides with the limit of normality in weight tono graphy (Duke Elder 1969)

5) Breaks defined as marked changes in the course of the experimental curve compared with the reference curves were observed both in narrow angled and open angled glaucomatous eyes or suspected eyes with facilities of outflow below 0 15 In three of four cases of narrow or closed angle glaucoma the polyphasic course was evident and the interpretation of this as resulting from the reopening of collapses is comprehensible from the cur rent ideas on the iris root mechanism of angle closure (Fig 2) originating from Barkan et al (1936) The phenomenon might also be explained by an unfolding of a collapsed Schlemm s canal however this structure was observed to be narrow in this type of glaucoma (Kessing 1971) The interpretation of the breaks observed in half of the curves from open angled eyes n the low facility group is not as straightforward because they cannot be judged on the

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GLUCOSE PYRUVATE L-LACTATE AND CITRATE CONCENTRATIONS IN THE AQUEOUS HUMOUR OF FASTING RABBITS IN RELATION TO AGE

BY

A BRUUN LAURSEN and S E. LORENTZEN

Results are reported of determinations of glucose pyruvate l lactate and citrate concentrations in aqueous humour from 42 fasting albino rabbits aged from 8 months to 9 years
The glucose concentrations decreased significantly with increasing age. The pyruvate and l lactate concentrations as well as the ratios of l lactate/pyruvate *did not change significantly with age However the pyruvate* concentrations were higher and the l lactate/pyruvate ratios were lower in the 2 year old rabbits than in the others The citrate concentrations were found to rise with increasing age
Because of wide ranges of variation between individuals of the same age groups the parameters in question have limited value in the evaluation of the energy metabolism of the anterior eye segment

Key words glucose – pyruvate – l lactate – citrate – aqueous humour – rabbits

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Glucose concentrations in heated samples and samples treated with NaF (ca 30 mmol NaF/l sample) from the same pool of aqueous humour were alike From each of six different aqueous pools 150 µl of aqueous was heated while another 150 µl of aqueous was transferred into 150 µl of 0.6 N $HClO_4$ (perchloric acid) The deproteinized aqueous $HClO_4$ mixture was then neutralized with 1.2 N KOH The concentrations of heated samples were expressed in percentages of the corresponding concentrations of the $HClO_4$ deproteinized samples The median percentages were pyruvate [illegible]9 % citrate 93 % and l lactate 109 %

Before determinations of pyruvate citrate and l lactate the rabbit aqueous humours were diluted with H_2O 1 + 1 because of the abundance of substrates

Methods

A Beckman glucose analyzer was used for glucose determinations (Kadish et al 1968) and a Beckman DB spectrophotometer for pyruvate, citrate, and l lactate determinations Measurements were performed at 340 nm in Helma 103 glass cuvettes with 10 mm light paths and at room temperature according to the procedures of Biochemica Boehringer with certain modifications

1 pyruvate citrate

Test cuvette 800 µl triethanolamine buffer 0.1 mol/l pH 7.6 (TEA) + 30 µl NADH 6 mmol/l + 50–100 µl aqueous + 350–300 µl H_2O Assay volume before addition of enzymes = 1230 µl *Blank* 800 µl TEA + 20 µl NADH 6 mmol/l + 400 µl H_2O Mix Record extinction Add 6 µl of lactate dehydrogenase suspension (LDH – catalogue No 15 371) to both cuvettes Mix. Record extinction till fall has stopped The fall in extinction is proportional to pyruvate concentration Add 6 µl of malic dehydrogenase suspension (MDH – catalogue No 15 048) Mix. Record extinction till it is stable. Add 6 µl of citrate lyase suspension (catalogue No 15 186) Mix Record extinction till it is stable The fall in extinction is proportional to citrate concentration (For principles of pyruvate and citrate determinations see Bruun Laursen 1973)

2 l lactate Test combination No 15 972

1000 µl hydrazine (0.1 mol/l) – glycine (0.125 mol/l) buffer pH 7.8 + 100 µl NAD 27 mmol/l + 230 µl H_2O + 20 µl aqueous Assay volume before addition of LDH = 1350 µl Mix Record extinction against a blank containing the same ingredients except for addition of 20 µl of H_2O instead of sample (aqueous) Add 15 µl LDH to both cuvettes Mix Record extinction until it is stable Rise in extinction is proportional to l lactate concentration. The principle of this determination is l lactate + NAD → pyruvate + NADH a process which is catalyzed by LDH This process measured at 340 nm causes rise in extinction

In this work the NADH concentrations of the cuvettes were reduced to ca. 150 µmol/l from ca 300 µmol/l in previous publications

The concentrations were calculated according to the formula (modified from Moellering & Gruber 1966) $\frac{\Delta A \times \text{assay volume (ml)}}{\varepsilon \times \text{sample (ml)}} = \mu\text{mol substrate per l aqueous}$ where ΔA = change in extinction and $\varepsilon = 6.22\ \text{mmol}^{-1}\ \text{l}\ \text{cm}^{-1}$ For recoveries see Table I

Table I

Recovery experiments for pyruvate citrate l lactate and glucose

Substrate	n	Number of days	Added µmol/l	Median recovered µmol/l	Range of variation µmol/l
Pyruvate in watery solution	29	[illegible]	300	292	[illegible]
Pyruvate in rabbit aqueous	14	3	100	91	15
Citrate in watery solution	29	7	100	91	[illegible]
Citrate in rabbit aqueous	14	3	100	92	30
	13	3	100	90	17
			mmol/l	mmol/l	mmol/l
Glucose in rabbit aqueous	10	2	5 6	5 5	0 6
l Lactate in watery solutions	19	5	3 0	[illegible] 4	0 5
	4	1	2 5	[illegible] 1	0 2
	5	1	1 0	0 8	0 1
l Lactate in rabbit aqueous	6	2	1 0	0 6	0 1

The added substrates were not heated Pyruvate citrate and lactate (3 0 mmol/l) determinations were performed on the same solutions which were stored frozen For pyruvate and citrate determinations on different concentration levels see Bruun Laursen 1975

Results

The results appear in Table II and Fig 1 Comparisons between two groups were carried out by means of the Wilcoxon test for two samples The age concentration correlations were tested by means of the Spearman coefficient of rank correlation

The glucose concentrations (median 5 2 mmol/l) were found to decrease with increasing age ($P < 0.001$) The median of 8 month old animals (six rabbits) was 5 9 mmol glucose/l whereas the median of the age group 63–104 months (12 rabbits) was 5 0 mmol glucose/l

The pyruvate concentrations did not change systematically with age (median

407 μmol/l – $P > 0.10$). At the age of 2 years, however, the pyruvate concentrations (median 477 μmol/l for six rabbits) were higher than in the other age groups ($P < 0.01$).

The l-lactate concentrations (median 5.2 mmol/l – not corrected for only 80 % recovery in watery solutions) did not change with age ($0.10 > P > 0.05$).

The ratios of l-lactate/pyruvate (median 13.5) did not change with age ($P > 0.10$) but Fig. 1 shows a lower level at the age of 2 years (median 10.4) than in the other age groups ($P < 0.01$).

The citrate concentrations (median 431 μmol/l) increased with increasing age ($P < 0.001$). While the median for 8 month old rabbits (six animals) was 345 μmol citrate/l, the median for 12 animals ranging in age from 63 to 104 months was 476 μmol citrate/l.

When considering the statistical levels of significance one must bear in mind the wide ranges of variation between individuals of the same age groups. The part played by the inaccuracies of the methods appears in Table I and the section on Material.

Discussion

In a previous paper (Bruun Laursen 1973) a survey was given of some reports from the literature on glucose, pyruvate, and citrate concentrations in rabbit aqueous humour. Table III gives some data on lactate concentrations in rabbit aqueous.

Because of variation with age, and possibly because the animals were fasting, the glucose concentrations of the present material (median 5.2 mmol/l) were lower than reported in the previous publication (Bruun Laursen 1973) where the mean was 6.1 mmol glucose/l.

As our recovery for l-lactate in watery solution is about 80 % – and we did not correct for the remaining 20 % – this may in part account for the fact that our lactate values are lower than those reported by Reim et al. (1969), Reddy & Kinsey (1960) and Riley (1972). The lower l-lactate concentrations in the present material (median 5.2 mmol/l) might also be because we examined fasting rabbits, and because we inactivated glycolytic enzymes immediately.

The pyruvate and citrate concentrations in this material are somewhat higher than previously found by Bruun Laursen (1973), who found the means to be 339 and 354 μmol/l respectively, against the present 407 and 431 μmol/l. This may be because for both parameters the recovery percentages in watery solutions increased after reduction of the NADH concentrations of the cuvettes to

Table II

Glucose pyruvate l lactate and citrate concentrations in rabbit aqueous humour Pyruvate l lactate and citrate concentrations were not corrected though recoveries in watery solutions were about 90 % 80 % and 90 % respectively

Age (months)	Glucose mmol/l	Pyruvate µmol/l	l Lactate mmol/l	Ratio of l lactate/ pyruvate	Citrate µmol/l
8	5 8	368	5 1	13 9	337
8	5 8	437	5 9	13 5	394
8	6 0	398	6 5	16 3	353
8	5 4	374	5 7	15 2	379
8	6 0	368	5 0	13 6	281
8	6 1	326	4 4	13 5	378
24	5 5	461	4 5	9 8	425
24	6 7	468	4 7	10 0	353
24	5 4	509	5 6	11 0	362
25	5 4	461	5 2	11 3	467
25	4 8	509	5 5	10 8	442
26	5 0	485	4 5	9 3	425
37	5 3	429	5 1	11 9	337
37	5 9	406	5 5	13 5	377
37	5 2	390	5 4	13 8	371
37	5 8	326	4 4	13 5	418
37	5 3	382	4 7	12 3	360
42	5 4	310	4 3	13 9	448
43	5 0	350	4 5	12 9	354
49	5 0	422	5 7	13 5	466
50	4 4	350	5 0	14 3	394
51	5 0	350	4 9	12 3	514
53	4 6	398	5 7	14 3	442
54	5 3	394	4 9	12 4	582
54	4 6	457	5 1	11 2	478

150 µmol/l This increase was about 10 % For citrate it is also of importance that *older age groups were represented in this material* as citrate concentrations increase with increasing age

Bruun Laursen (1973) found no age dependence for pyruvate or citrate in the aqueous humour of non fasting rabbits aged 4–36 months However the ratios of pyruvate/citrate did decrease significantly with increasing age (0 05 > P >

Table II (cont)

	Age (months)	Glucose (mmol/l)	Pyruvate (μmol/l)	l Lactate (mmol/l)	Ratio of l lactate/ pyruvate	Citrate (μmol/l)
	58	4 7	366	5 1	13 9	433
	58	5 2	479	5 6	13 1	603
	58	5 0	433	5 2	12 0	510
	58	5 3	492	5 1	12 1	510
	59	5 0	370	4 7	12 7	453
	63	4 4	350	4.2	12 0	462
	63	5 4	425	6 0	14 1	634
	64	5.3	473	5 7	12 1	469
	64	4 9	413	5.3	12 8	450
	64	5 7	361	4.5	12.5	506
	69	4 7	433	5 8	13 4	369
	69	5.2	425	6 2	14 6	499
	69	4 7	461	6 7	14.5	514
	71	4 9	339	6 1	15 7	499
	75	5 0	362	4 9	13.5	482
	95	5 2	4 6	5 8	13 6	502
	104	4 7	409	6.3	15 4	589
n = 49						
Medians	50 1/2	5 2	407	5.2	13 5	431
10 % percentiles	8	4 6	350	4 4	10 9	337
90 % percentiles	0	6 0	472	6 1	15 0	562
Ranges of variation	96	2.5	199	2.5	[illegible] 0	353

0 02) In cattle Bruun Laursen (1972) found falling pyruvate and rising citrate concentrations with increasing age

The increase of citrate concentrations of the aqueous humour with increasing age may be due to inhibition of the tricarboxylic acid cycle or it may be effected by increasing demands on the oxydative metabolism of the surrounding tissues This problem so far remains unsolved The individual citrate concentrations of

Table III

Some data on lactate concentrations in rabbit aqueous humour from the literature

Author	Year	Number	Fasting	Lactate concentrations (mmol/l)
Auricchio & de Vincentiis	1951	5	?	2 8–8 1
Bito & Salvador	1970	≧ 6	?	2 93
Reddy & Kinsey	1960	pooled	?	anterior 9 3 posterior 9 9
Reim et al	1969 1972	21 14	?	7 45 ± 0 34 8 5 ± 0 7
Riley	1972	10	?	anterior 9 9 ± 1 6 posterior 9 0 ± 1 6
Bruun Laursen & Lorentzen	1974	42	+	5 2 (l lactate) (the true value is probably 6 2)

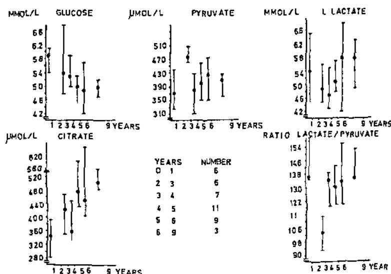


Fig 1

Glucose pyruvate l lactate and citrate concentrations in the aqueous humour of fasting albino rabbits in relation to age The black dots indicate the medians of each ag group and the vertical lines give the range of variation of each age group

The numerals under the heading Number refer to the number of rabbits in each age group

the aqueous seem to be widely independent of plasma citrate concentrations (Bruun Laursen (1972) – cattle Bruun Laursen (unpublished) and Bruun Laursen & Lorentzen (1974) man)

The age dependencies of glucose and citrate in aqueous render these parameters less fit for estimating the energy metabolism of the anterior eye section in random samples from a rabbit population The ranges of variation between individuals of the same age groups are especially wide for l lactate and for the ratios of l lactate/pyruvate (median 13 5) see Fig 1 This diminishes the value of these parameters in relation to evaluation of the energy metabolism of the anterior eye section

The question arises as to whether the recorded changes with age are due to changes in one or more tissues in contact with aqueous or whether they might be due to similar changes in plasma In this context it should be mentioned that with increasing age Pohjola (1966) found decreasing ratios glucose in aqueous/glucose in plasma in normal human eyes

We examined the ratios of glucose in arterial plasma (heparinized and fluoridated)/glucose in aqueous in eight fasting rabbits aged from 52 to 71 months (nembutal anaesthesia) and we found values ranging from 1 1 to 2.3 without age dependence This parameter must therefore be considered unfit for the evaluation of the energy metabolism of the anterior eye section of rabbits

Reim et al (1969) found pyruvate and lactate concentrations in rabbit aqueous to be ca four times higher than the corresponding plasma concentrations indicating that changes in plasma concentrations of these metabolites will only slightly influence the aqueous levels

Bruun Laursen (1972) demonstrated age dependence for citrate concentrations in cattle aqueous humour whereas no age dependence could be demonstrated for the corresponding plasma citrate concentrations

Since age dependence of certain metabolites in the aqueous humour of rabbits and oxen has been demonstrated it might be interesting to look for possible similar changes in the aqueous in connection with human senile cataract development as cataract passes from immature stages to the stage of total lens opacity A preliminary report on this topic was given by Bruun Laursen & Lorentzen (1974) and will be dealt with an greater detail in another paper (unpublished)

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NORMAL VALUES IN CLINICAL ELECTROOCULOGRAPHY

I Material Method Methodological Investigations and Distribution of the Potential and Time Parameters

BY

ERIK KROGH

For the evaluation of normal EOG potential and time parameters a case series (147 eyes) is presented An EOG technique aiming at the largest possible amplitude between the dark trough and the light peak is described It is shown that the light induced response following a 20 min period of adaptation to a very low degree of illumination (about 1/10 lx allowing a rough orientation on the displayed trace) does not differ from that produced after 20 min of total darkness Also the independence of the pupillary area for the light induced response is demonstrated which means that the light stimulus employed ([illegible]500–4000 lx) is supramaximal Furthermore the good quality of the gaze fixation in the actual test situation with respect to the recording of equidistant saccades is proven. A DC amplification of the signal is used which secures independency of inter and intra individual differences in the saccadic velocity and makes possible accurate measurements of deflections disfigured by correcting movements The following EOG parameters are recorded A base value after 10 min of preadaptation with the light stimulus the dark trough and the light peak potentials Also the periods between the beginning of the dark adaptation and the dark trough and between the dark trough and the light peak are noted The frequency distribution and the general level of the various parameters are discussed in the light of comparable figures of previous publications

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Key words EOG – electrooculography – corneofundal potential – electro physiology – DC recording

The clinical value of the indirect recording of the corneofundal potential (CFP) and its oscillations (electrooculography EOG) has been under dispute since the promising reports of François & de Rouck (1955) François et al (1955 1956a 1956b 1957) and Arden et al (1962) The large dispersion of the test parameters is evident and investigations focused on this aspect conclude that at present the EOG is an unsuitable tool for diagnosis and follow up of pathological processes in the individual patient (Kelsey 1967 Muller & Haase 1970 Hochgesand & Schicketanz 1974)

The reasons for this dispersion have as yet not been satisfactorily elucidated. Significant contributions probably come from the inevitable variation of the distance between the potential generator and the skin electrodes (Mackensen & Harder 1954) and the shunting effect of the peribulbar tissues whose conductance cannot be expected to show strict concordance from person to person and perhaps not even a sufficient intratest stability

A condensor coupled (AC) transfer system with a too small time constant in comparison with the rise time of the signal introduces another source of error The rise time of a 30° saccade varies from 0 05 to 0 3 s according to velocity studies by Kris (1960) and Boghen et al (1974) For such a ramp function the ratio time constant/rise time should exceed 50 in order to reduce the amplitude loss in the amplifier input to the 1 % level (Sten Knudsen & Nissen Petersen 1971) A comparison between AC and DC recordings of 30° saccades shows that even a time constant of 5 s involves an amplitude loss of about 10 % which additionally is liable to inter as well as intratest variability (Krogh 1975)

Different and possibly age and sex biased test samples holding a varying number of retests impair any joint conclusion regarding the normal values of the EOG parameters from the literature and possible correlations between the EOG and other parameters have not been systematically studied

The aim of the present and following papers is therefore to describe an EOG test procedure in particular characterized by DC amplification and its outcome in a normal case series with well defined ophthalmological as well as age and sex parameters

This paper describes the material the EOG technique and the general distribution of the collected data Furthermore the practical significance of both the depth of the initial dark adaptation and of the pupillary area for the magnitude of the subsequent light rise will be estimated just as the accuracy of the gaze fixation under the test circumstances will be subjected to trial

Collection and Description of the Material

The normal case series was collected from among patients hospitalized for minor local diseases in the departments of orthopedic surgery plastic and maxillofacial surgery and ENT diseases Rigshospitalet, Copenhagen. All patients in a 3 month period during the winter 1973 were considered for inclusion except that towards the end of the period a suitable age and sex distribution was aimed at

Patients with current eye diseases or earlier intraocular bilateral pathology as well as metabolic hormonal neurological and circulatory disturbances malignancies and connective tissue disorders were excluded without further investigation. In the preliminary ophthalmological examination the following criteria for inclusion were used a) A corrected visual acuity of at least 6/9 and a refraction not exceeding ± 5 00 dp sph In case of astigmatism the limits were ± 6 00 dp sph equivalent, the largest difference allowed for being 2 00 dp In Fig 1 the distribution of the values of the refraction (without cycloplegia) is depicted b) Normal orbital regions normal position and motility of the eyes c) Normal findings (including physiological age variations) by slit lamp examination of the anterior part of the eye and by ophthalmoscopy d) Normal examination at the Bjerrum screen (white 5/1000) and an applanatic pressure of 22 mm or less

Seventy two persons passed the screening and carried through the following procedures including the EOG without remarks 1) Examination with the Javal Schiotz ophthalmometer (Haag Streit) 2) Measurement of the horizontal diameters of the cornea and the pupil in the Goldmann perimeter with a 45 lx illumination 3) Measurement of the interpupillary distance with an Oculus DIP meter 4) Measurement of the ocular protrusion (Rodenstock a m Hertel) 5) Estimation of the iris pigmentation graded into four classes according to a scheme by Tocher (1908) 6) Measurement of the axial length

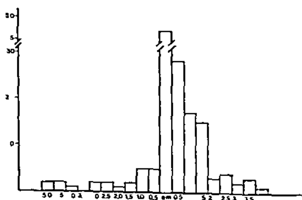


Fig 1

The non cycloplegic refraction values of the 142 eyes comprising the normal case series Cases of astigmatism are enumerated with the spherical equivalent The figures under the columns are the upper limits of the 0.5 dp class interval

of the eye with an ultrasound technique described in detail by Fledelius (1970) the frequency distribution is shown in Fig 2 The age and sex distribution of these selected test persons is given in Table I All were two eyed but with respect to the criterion of normality one eye with an optimal aphakia and another completely restored after a scleral perforation 2 years ago were not included

Method and Methodological Problems

The recording was performed in a Faraday cage with ceiling and walls of matt light grey colour The person under test was placed comfortably in the supine position Head movements were fully controlled with a surgical head rest pillow Guided by a metronome (about ½ Hz) the person fixated alternatingly two small dark red bulbs separated by a distance corresponding to a visual angle of 30° The accuracy of the gaze fixation

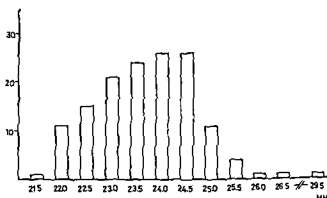


Fig 2
The axial lengths of the 142 eyes comprising the normal case series The figures under the columns are the lower limits of the 0 5 mm class interval

Table I
Age and sex distribution of the 72 persons comprising the EOG normal case series

Age groups	10–19	20–29	30–39	40–49	50–59	60–69	70–81	Total
Females	5	4	3	6	4	7	7	36
Males	3	8	0	9	6	[illegible]	5	36

under these circumstances was tested in 20 persons selected from among the staff and ward patients of the eye clinic some deliberately chosen with subnormal visual acuity (lower limit 6/18) Circles of white paper with a diameter corresponding to a visual angle of 1° were placed around each fixation light An after image of a cross (vertical bar in one eye horizontal in the other) was produced by a modified Braun photoblitz The test persons were told to move their eyes from one fixation light to the other guided by a metronome (0 ? 1 Hz) under illumination conditions varying as in the EOG test All test persons placed the centre of the cross within the 1° visual angle area, eventually after a swift over or undershoo Most of the test persons invariably placed the centre at the same site each time usually at the transition between the bulb and the paper The shape of the cross was unaltered by the version indicating a symmetrical movement of the two eyes

Transfer system

The electrodes employed were 12 mm flat discs of a lead alloy (Kaiser Laboratory Copenhagen) fastened to the degreased and Ringer imbibed skin medial to the internal canthus on the nasal bone, and lateral to the external canthus on the zygomatic bone The contact medium was bentonite paste

The amplification and display were performed with a Mingo graph M 34 (Siemens) provided with EMT 12 B preamplifiers with an input resistance of 50 MΩ The gain was sele ted in the 20–40 μV/mm range giving rise to deflections from 4 to 45 mm (effective record span 84 mm) and the paper speed was 10 mm/s The bandwidth was 0–15 Hz securing a DC recording of the lowest possible noise level The value of the upper cut off frequency (corresponding to a time constant of 0 01 s) signifies that even a deflection produced by the fastest 30 saccade (rise time 0 0? s) will reach the correct potential level with a delay of less than 1 rise time (Sten Knudsen & Nissen Petersen 19 1) The electrical characteristics of the transfer system and the precautions to be taken in a DC recording have been examined (Krogh 1975)

Ten twenty saccades were recorded every min Some of the older persons were only able to perform usable saccades every second min especially in the beginning of the light adaptation

Stimulus Conditions

According to Arden et al (196) the light induced (or more correct the light amplified) response of the dark adapted eye constitutes the EOG parameter which is most sensitive for pathological processes in the posterior half of the eye. Consequently the present EOG procedure was constructed as an attempt to maximize this parameter

Preadaptation

The intensity of the illumination does not influence the level (the dark trough) to which the potential drops after extinction of the light (Arden & Kelsey 1962a Muller et al 19 0) Nevertheless to be able to compare the present material with that of Gliem

(1971) a 10 min preadaptation with the light stimulus initiated each test. The potential value obtained at the end of the preadaptation was termed the base value

Dark adaptation

When the intention is to obtain a maximal light induced potential response the length of the dark adaptation must be sufficient for the development of a dark trough which usually occurs about 10 min after the extinction of the light Arden & Kelsey (1962a b) seem to conclude that further augmentation of the light induced response can be expected with up to 20 min of dark adaptation but their figures on this point are not clear

For the development of the dark trough only a certain reduction in illumination intensity ($\geq$ 1 69 log units) is required (Arden & Kelsey 1962a Gliem 1971) but for the magnitude of the following light rise the importance of the reduction factor has not been laid down although the practical implications are obvious in simultaneous recordings from both eyes or in recordings from a single useful eye where a tight bandage would interfere with the accuracy of equidistant eye movements in the same plane Therefore it was tested whether any difference in the light induced response could be recorded between a 20 min period of total darkness and a 20 min period of very weak illumination coming from the fixation light and the recording equipment allowing a rough orientation on the ink trace (1/10 lx in the pupillary plane of the test person) Two pairs of EOG s were recorded from the same eye in four test persons (healthy medical students) In the first pair the dark period of the initial EOG was secured by a tight bandage and after 2–3 hours the EOG was repeated with the unbandaged eye adapting to the above mentioned weak illumination In the second pair of EOG s recorded a week later the order was reversed without disclosing any systematic differences As an expression for the light induced response the Arden ratio (light peak/dark trough) was used and a Mann Whitney test did not demonstrate any differences of the ratios obtained with the two test procedures ($P > 0\ 1$)

In the pre ent test the dark period (1/10 lx) was not terminated before the dark trough was passed in both eyes which can be difficult to ascertain during the test In cases of doubt the dark period was therefore extended to 20 min The shortest dark period was 14 min

Light stimulus

Arden & Kelsev (1962a) demonstrated a linear relationship between log stimulus intensity and amplitude of the light induced response for intensities up to 10 000 trolands beyond which limit no further increase of the light induced response could be recorded. Correspondingly Muller et al (1971) found that stimuli of 800 and 1200 lx did not give rise to significant differences in the light induced response irrespective of the magnitude of the pupillary area the troland range being 15 270–66 740

The light stimulus in this study was provided by an Osram 2000 W halogene tube with a colour temperature of 3200 K The light was diffused by an opaque screen mounted in front of the lamp house The distance between the person under test and the lamp was selected to provide an illumination from 4000 lx in the central part of the gaze field to 2500 lx in the periphery measured at the pupillary plane (H & B luxmeter Kemp & Lauritzen)

According to the above mentioned authors the light stimulus applied in the present study should be supramaximal but for confirmation the light induced response with different pupillary areas was recorded and compared in eight test persons (healthy medical students) One EOG was performed with freely reacting pupils followed by a retest with the left pupil pharmacologically constricted (four persons) or maximally dilated (four persons) The right eye served as a control A Mann Whitney test showed no significant differences between the Arden ratios in the first and in the second performances either in the left or in the right eyes ($P > 0.1$)

When the light was turned on the potential was followed until the light peak was passed In a few cases the stimulus was changed to ordinary room illumination after 5–6 min because of complaints from the person under test (the study of Gliem (1971) demonstrated that an exposure time of 4 min is sufficient to elicit a full light induced response)

Evaluation of the Trace

The deflections were measured in mm with one decimal with a slide gauge (Mitutoyo Diamond) with 0 05 mm divisions The measuring error of the single deflection was estimated to ± 0 1 mm (range of deflections 4–45 mm) The mean value of each group of usable deflections (7–18) was calculated (1 decimal) and transposed to a potential value (μV zero decimals) according to the calibration

Three recordings were excluded on account of bad quality in the form of noisy and drifting traces not influenced by reapplicating the electrodes securing the cable connections changing the position of the amplifier or by expectation (In two recordings the derailing influence of the drift could be controlled by a considerable diminution of the gain, but as this brought about very small deflections – less than 4 mm which with reference to the measuring error seemed a suitable lower limit – the traces were nevertheless not included in the series)

No supported statement regarding the physiological and clinical significance of the EOG time factors is available For comparison the periods between the extinction of the light and the dark trough and between the dark trough and the light peak were measured in min.

For recapitulation the present EOG procedure consists of the following steps 1) Ten min of preadaptation with the light stimulus 2) Reduction of the illumination to almost darkness (1/10 lx) followed by the recording of 10–20 30° saccades every min until the dark trough is passed 3) Reapplication of the light stimulus (2500–4000 lx) and recording as under (2) until the light peak is passed A standard trace and its transformation are depicted in Fig 3

Resulting EOG Parameters

Base value

In 31 of the 142 eyes it was not possible to record a base value due to large DC potential differences which however diminished sufficiently with time so as to

use of small electrodes placed very close to the canthi which is not advisable in a DC amplification (Krogh 1975)

The potential levels of Gliem (1971) are situated somewhere between the levels of the present study and those of Arden & Barrada (1962) The electrode placing seems comparable to that of Arden & Barrada (1962) but the time constant of the amplifier is only 0 3 s

The EOG time factors in the present study compare well with those of Arden & Barrada (1962) and Gliem (1971)

The possible disadvantages of the DC amplification need a comment The 31 lost base values could have been recorded with better means of DC compensation than at disposal The three discarded curves could probably have been recorded with an AC amplification with a suitably small time constant but even in this case there is no guarantee for a clean recording free of high frequency noise From the preceding discussion it is seen that it is possible to secure higher EOG potential levels with an AC technique compared to the present DC technique but that their accuracy and precision inter as well as intraindividually are open to question

Acknowledgements

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Each subject reported in the morning prior to inserting his lenses and a measurement of corneal touch threshold was made subjectively (Millodot 1973) in the centre and in a peripheral point (6 o clock) of the cornea The measurements began with stimulation of the cornea at the lowest pressure and continued in an ascending fashion At each predetermined length (in increments of one half a cm) of the monofilaments four to six contacts were made and the slightest bend of the nylon wire visible through a X 4 3 magnifier was defined as corneal contact The subject was fixating an object on the opposite wall and he indicated when he felt the probe by pressing a bell From these readings the touch threshold was defined as the length of the monofilament for which the subject responded for 50 % of the number of stimulations This length was converted into pressure using a previously established calibration curve between length and pressure Corneal thickness was also recorded with the pachometer following the instructions provided with the instrument

The subjects were then asked to report to the laboratory after having worn their lenses continuously for 8 hours A measurement of corneal touch threshold was carried out within 1 min of removal It was followed by other readings 4 7 10 15 20 25 and 30 min after removal The measurements of central and peripheral corneal sensitivity and of central corneal thickness were each carried out on a different day after the subject had kept his or her lenses for 8 hours

Results

The effect of continuous wear of hard contact lenses for 8 hours on corneal sensitivity (threshold [1]) is illustrated in Fig 1 The upper curve (continuous line) represents the peripheral measurements and the lower curve (broken line) the central measurements Each data point is the mean of 11 subjects The mean corneal touch threshold before inserting the lenses in the morning was 13 86 mg/sq mm (s d 4 42) and 19 64 mg/sq mm (s d 4 90) in the centre and periphery respectively The well known difference between centre and periphery is again confirmed (Boberg Ans 1955 Cochet & Bonnet 1960 Millodot & Larson 1969)

After 8 hours wear the corneal touch threshold was found to be 25 91 mg/sq mm (s d 7 88) and 45 64 mg/sq mm (s d 17 5) in the centre and periphery respectively Both these values are significantly higher ($P < 0\ 001$) than they were prior to insertion The threshold in the corneal periphery increased on average by 116 % (range from 50 to 200 %) and by 94 % (range from 34 to 200 %) in the centre This decrease in sensitivity after wearing hard lenses is in qualitative accord with other studies

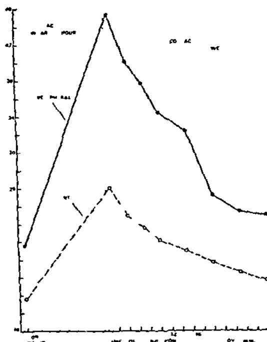


Fig 1

Relationship between central and peripheral corneal touch threshold (sensitivity^{-1}) and the wearing of hard contact lenses for 8 hours. Each data point is the mean of 11 subjects

The loss of corneal sensitivity as found in this study is less in the centre than in the periphery. This fact may be accounted for by the following consideration. When testing the sensitivity in the centre of the cornea, subjects are usually apprehensive at first and therefore the threshold obtained is lower. After a few minutes of testing they relax and their threshold is less dependent on apprehension, a phenomenon which has already been demonstrated (Bonnet & Millodot 1965). This factor would contribute to the reduction of the height of the peak of the broken curve in Fig. 1.

After removal of the contact lenses there is a progressive decline in corneal touch threshold (i.e. an increase in sensitivity) which is greater within the first 20 minutes and diminishes more slowly afterwards. Thirty minutes after removal the corneal touch threshold was found to be 15.82 mg/sq mm (s.d. 4.59) and 22.3 mg/sq mm (s.d. 5.46) in the centre and periphery respectively. These values are significantly lower ($P < 0.001$) than those found immediately after removal. Although these values are close to the thresholds obtained in the morn-

ing when the subjects have not worn their lenses overnight for some 10 to 12 hours the differences are nonetheless significant ($P < 0$ 01) which means that corneal sensitivity has not fully recovered within 30 min Yet it has regained 82 and 100 % (for the centre and periphery respectively) of what it had lost during the 8 hours of wear

The effect of continuous wear of hard contact lenses for 8 hours on central corneal thickness is illustrated in Fig 2 Each data point is the mean of 11 subjects The mean corneal thickness before inserting the lenses in the morning was 0 520 mm (s d 0 033) This value is in good agreement with the result of other authors (see Hansen 1971) After 8 hours corneal thickness increased to 0 556 mm (s d 0 041) This result is significantly higher ($P < 0$ 001) than before inserting the lenses Thus the corneal thickness increased on average by 6 9 % (range from 1 to 12 %) This finding is in good qualitative accord with the results of the investigations cited earlier In only one subject was there only a very slight increase (1 %) in corneal thickness but there was nevertheless a decrease in central (34 %) and peripheral (50 %) corneal sensitivity *On the* other hand the subject displaying the greatest increase in corneal thickness had also the largest reduction of corneal sensitivity

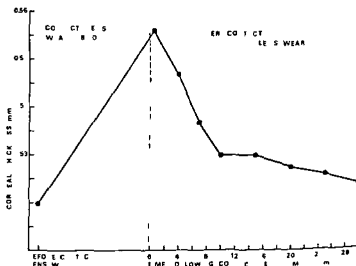


Fig 2

Relationship between central corneal thickness and the wearing of hard contact lenses for 8 hours Each data point is the mean of 11 subjects

Thirty minutes after removal corneal thickness decreased to 0 524 mm (s d 0 037) which is significantly ($P < 0.001$) lower than immediately after removal The decline of corneal thickness after 30 min was on average 6 3 % Although the mean corneal thickness 30 min after removal is very close to the mean corneal thickness in the morning it is significantly different ($P < 0.05$) which means that corneal thickness has not fully recovered within that time

A comparison between central and peripheral measurements of corneal sensitivity obtained in the morning prior to inserting the lenses yielded a correlation coefficient of 0 78 which is significant ($P < 0.01$) Thus it appears to be valid to evaluate the effect of contact lenses on corneal sensitivity by measuring either area of the cornea

Discussion

This study shows that hard contact lenses cause a significant reduction of corneal sensitivity and a significant increase in corneal thickness at least after 8 hours of contact lens wear Qualitatively similar effects were shown after the wear of soft contact lenses (e g Bailey & Carney 1973 El Hage et al 1974 Millodot 1974a) but the effects were far less marked than with hard contact lenses After 8 hours of hard contact lens wear the loss of sensitivity is such that it would seem wise to advise patients to remove their lenses for 20 to 30 min since the cornea recovered most but not all of its sensitivity and initial thickness within that period of time These results are in good agreement with the work of Farris Kubota & Mishima (1971) who found that corneal oxygen uptake diminished some 10 min after contact lenses which had been worn for 8 hours were removed

However corneal sensitivity and thickness take more than 30 min to recover completely as the data obtained in the morning were significantly lower than 30 min after removal Theoretically sensitivity should continue to increase beyond the level of the morning measurement as it is known to increase during the day in people who do not wear contact lenses (Millodot 1972) and similarly corneal thickness decreases somewhat throughout the day (Gertsman 1972) Hence it can be inferred that the effect of hard contact lenses on the cornea is even greater than is evident from the present data

Hard contact lenses are supposed to interfere with corneal metabolism by changing tear osmolarity and by oxygen deprivation As the subjects of the present investigation were well adapted to their contact lenses it has to be assumed that the increase in corneal thickness was caused mainly by oxygen

deprivation This is because tear osmolarity has been shown not to change significantly after 8 hours of contact lens wear in well adapted subjects (Farris Kubota & Mishima 1971) Thus oxygen deprivaton leads not only to oedema which is known to be related to corneal thickness (Ytteborg & Dohlman 1965 Mishima & Hedbys 1968) but also to a large reduction in corneal sensitivity It is not yet known how oedema is related to a diminution of corneal sensitivity but the present study emphasises the fact that these two parameters are affected simultaneously as a result of contact lens wear

Corneal thickness is an index of corneal oedema but one does not infallibly follow the other as Farris Kubota & Mishima (1971) have shown by assessing the oedema by sclerotic scatter illumination This lack of perfect correlation must stem in part from the difficulty encountered in obtaining these measurements accurately and reliably The change in corneal thickness found in the present study was 6 9 % on average Such measurements have to be extremely accurate to be significantly above experimental error On the other hand the change of corneal sensitivity occurring with the same stimulation amounted to above 100 % on average thus making this measurement a far more practical index of corneal integrity

The measurements of corneal sensitivity are easy to obtain and with far less expensive equipment than a slit lamp and a pachometer It is more reliable to measure the sensitivity of a peripheral corneal point such as at 6 o clock because psychological factors are absent as compared to the testing of central corneal sensitivity (Bonnet & Millodot 1965 Millodot 1974b) And since it was shown in this study that peripheral and central corneal sensitivity are well correlated it is a valid procedure Moreover it was shown that hard contact lenses produce a decrease of central as well as peripheral corneal sensitivity and this result is in accord with the fact that corneal thickness in the centre and periphery was found to increase after contact lens wear (Beaulne 1974)

Finally it must be noted that unlike corneal thickness measurements or sclerotic scatter observations corneal sensitivity is a subjective method of assessing corneal integrity Thus it complements the objective methods and clinical tradition has it that objective methods are often to be compared against the presumably true subjective determinations

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HISTOLOGY OF THE IRIS IN GEESE AND DUCKS PHOTOSENSITIZED BY INGESTION OF AMMI MAJUS SEEDS

BY

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A SHLOSBERG*** and A EILAT***

Geese and ducks were photosensitized by the ingestion of *Ammi majus* seeds and exposure to sunlight Mydriasis was a characteristic clinical feature of this syndrome in both species Histologically the iris of the affected birds showed vacuolisation and varying degrees of atrophy of the muscle of the sphincter pupillae The effect of pilocarpine and physostigmine on the normal and mydriatic eyes was studied The possible mode of action of photosensitization and the significance of these findings in the light of the use of psoralens in human medicine is discussed

Key words geese – ducks – photosensitization – methoxypsoralen – *Ammi majus* – mydriasis iris atrophy – pilocarpine – physostigmine – psoriasis – vitiligo

Photosensitization induced by the ingestion of the plant *Ammi majus* caused severe ocular symptoms in geese in field outbreaks (Egyed et al 1974a and Eilat et al 1974) and in ducklings under experimental conditions (Egyed et al

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1975) Mydriasis was one of the most striking ocular manifestations and in this study we describe its histological basis

Material and Methods

Geese which had been involved in a field outbreak of *A majus* induced photo sensitization (Eilat et al 1974) were examined and birds showing different degrees of mydriasis were sacrificed and their eyes examined histologically

Photosensitization was induced in 3 week old ducklings by force feeding with *A majus* seeds for 5 days in conjunction with exposure to sunlight (Egyed et al 1975) These birds as well as clinically healthy ducklings serving as controls were sacrificed 2 weeks 3 1/2 months 5 1/2 months and 10 months after the beginning of the experiment and their eyes examined histologically

Processing of the eyes was carried out by standard methods Sections were *stained with haematoxylin and eosin* PAS *and* Masson*s trichrome techniques*

In order to investigate the functional state of the iris sphincter muscle drops of 6 % pilocarpine nitrate and 0 25 % physostigmine salicylate were instilled into the eyes of photosensitized and control birds 4 to 5 times at intervals of 3 min Examination of the eyes was performed under identical conditions of illumination

Results

Histology

Histological findings in the iris were basically similar in both geese and ducks In acute cases of photosensitization 2 weeks after the ingestion of *A majus* seeds the muscle bundles of the sphincter pupillae of the ducklings showed vacualisation only (Fig 1) In chronic cases of photosensitization in both geese and ducks varying degrees of atrophy of the sphincter were observed at the pupillary area (Fig 2) Severe atrophy was in some cases associated with ectropium uveae (Fig 3) The ciliary portion of the sphincter muscle was usually less affected than the pupillary area and showed in some cases a moderate vacuolisation only Geese with partial mydriasis exhibited atrophy of the sphincter muscle only at the ventral portion whereas geese and ducks with total mydriasis showed atrophy both at the dorsal and ventral segments

The dilator muscle fibers had disappeared in cases of severe atrophy of the sphincter pupillae associated with ectropium uveae (Fig 3)

Effect of pilocarpine and physostigmine on the pupil

In geese both pilocarpine and physostigmine elicited a moderate contraction of the pupil in both normal and photo sensitized birds with partial mydriasis The total mydriatic pupil did not react to either drug

In ducks pilocarpine drops were ineffective and physostigmine drops caused death within 20 min in both normal and photosensitized birds

Discussion

Photosensitization induced by *A majus* can be attributed to the action of 5 and 8 methoxypsoralen two potent photodynamic agents found in the seeds of this plant the latter of which has been widely used in the treatment of vitiligo (Fitzpatrick & Pathak 1959) Recently 8 methoxypsoralen has been used in the treatment of psoriasis initially as a topical preparation (Walter & Voorhees 1973 Weber 1974) and later with more success as an oral preparation (Parrish et al 1974) Pigmentary retinopathy was observed in *A majus* photosensitized ducks (Egyed et al 1975) and in ducks photosensitized by the administration of the medical preparation of 8 methoxypsoralen itself (unpublished data) All these photosensitized ducks exhibited a total mydriasis which developed in less than 2 weeks Mydriasis was seen in 13 % of photosensitized geese in a field outbreak induced by the ingestion of *A majus* (unpublished data) About 60 % of these geese exhibited a total mydriasis and the remainder only a partial mydriasis The exact time of appearance of the mydriasis was not determined In all affected geese and ducks mydriasis was present in both eyes to the same degree In eyes with total mydriasis the pupil was uniformly round the iris was reduced to a narrow rim and there was no pupillary reaction to light. In eyes with partial mydriasis the pupil was displaced ventrally (downwards towards 6 o clock) and the iris reacted to light with a moderate contraction (unpublished data)

The atrophy of the muscle of the sphincter pupillae with its subsequent ectropium uveae is probably the histological basis of the mydriasis The vacuolisation and atrophy of the sphincter muscle of the iris can be explained by the phototoxic effect of the light activated 5 and 8 methoxypsoralens which were presumably present in the iris after distribution by the blood stream in the whole body The same phototoxic effect was manifested in the skin of these birds as a severe necrosis (Shlosberg et al 1974) The difference in severity of the pathological changes between the pupillary and ciliary portions of the iris may be explained by the reduction in size of the palpebral aperture at the acute

C

Fig 1

I E

Fig 2

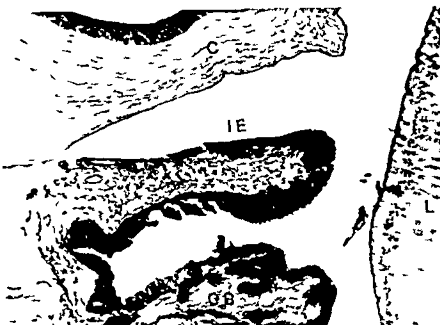


Fig 3

Abbreviations

C Cornea I Iris L, Lens v Vacuole I E Iris epithelium C B Ciliary body

stage of photosensitization In these eyes light could reach only the pupillary area of the iris and initiate the phototoxic reaction only in this region

Our experiments with pilocarpine and physostigmine confirmed the presence of remnants of functioning sphincter muscle in the irises of geese with partially

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Fig 1
E P ?408 1 month old duckling Two weeks after photosensitization with *Ammi majus* seeds Vacuolisation in the muscle of the sphincter pupillae × 120

Fig 2
E P ?1/3 11 month old duck Ten months after photosensitization Extreme atrophy of the sphincter at the peripupillary area The iris epithelium is bare. × 1 0

Fig 3
E P 178 6 month old goose Field outbreak Atrophy of the iris and ectropium uveae × 1?0

With about 5 mill inhabitants and a birth rate of less than 1 50 only a few cases of retinoblastoma can be expected every year in Denmark Despite this numerical paucity the retinoblastoma has been extensively studied The natural history was discussed by Bech & Jensen (1961) and Jensen (1965) In the latter publication the histopathological aspects were particularly emphasized The prognosis was studied by Kindt (1939) and the occurrence in a 48 year old man was reported by Rasmussen (1944) Spontaneous regression was discussed by Nehen (1975) while a partial spontaneous regression and necrosis of the central retinal artery was reported by Andersen & Jensen (1974) The treatment by irradiation was discussed by Boberg Ans (1958) and its organizational aspects by Ry Andersen (1971) while hereditary aspects of retinoblastoma were evaluated by Nielsen & Goldschmidt (1968) and Warburg (1974) Recent papers from the other Scandinavian countries were published by Jerndal Lindstedt Svensson & Akerskoe (1973) Jerndal & Tengroth (1974) Hørven (1974) Tarkkanen & Tuovinen (1971)

Material

In the period 1953–1973 a total of 45 children with retinoblastoma were treated at the Department of Ophthalmology and the Radium Centre Århus Kommunehospital University of Aarhus This series comprised 27 bilateral and 18 unilateral cases The relatively high number of bilateral cases is caused by the centralization of these cases from the whole of Denmark while the unilateral cases all originated from the local area of the hospital Ten of the 27 bilateral cases were girls while among the 18 unilateral cases seven were girls Among the unilateral cases eight occurred in the right and ten in the left eye Among the bilateral cases the right eye was primarily affected in 14 cases the left in 13 cases The localization in the eye could be determined in 53 tumours 13 of which occurred in the posterior pole the remainder in the periphery There was no tendency to concentration of the tumours in any particular quadrant

The average age at the time of the first symptom was for the bilateral cases 8 7 months as compared to 24 3 months in the unilateral cases The distribution of the series according to age at first symptom and at first admission is shown in Table I The range of ages at first symptom was for the bilateral cases 0 5–36 months for the unilateral cases 3–53 months In eight cases multiple tumours occurred in one eye

Techniques of radiation treatment

The radiosensitivity of the retinoblastoma was observed in 1903 by Hilgartner and subsequently Moore (1929) and Martin & Reese (1936) succeeded in destroying the tumour by irradiation Since then radiotherapy has improved and today several possibilities are available either the application of radioactive material adjacent to the tumour or external radiation can be used alone or in combination with cytostatics Additional therapeutic measures comprise coagulation of the tumour by light by cold or by diathermy Radiotherapy and cytostatics both depend upon a persistent vascularization

Table I
Age distribution for 45 cases of retinoblastoma at onset of symptoms and at first admission

Age groups	Bilateral		Unilateral	
	at onset of symptoms no	at first admission no	at onset of symptoms no	at first admission no
0–5 months	16	9	4	3
6–11 months	[illegible]	5	2	2
1–2 years	4	9	3	1
2–3 years	2	4	7	10
3–4 years	–	–	1	1
4–5 years	–	–	1	1

The large number of bilateral cases is caused by the centralized treatment of these during the last 20 years

Radioactive applicators This treatment was initiated by Moore (1929) who inserted radon needles into the tumour Modifications of this technique were in use until Stallard (1948) introduced his shell formed radium applicators which have the same curvature as the sclera and are sutured to the sclera outside the tumour The construction of these applicators was discussed by Williams (1957) and the calculation of isodose curves by Raimondi (1971) The applicator was modified by substituting ^{60}Co for the original radium preparation A slightly different technique was introduced by Rosengren & Tengroth (1963) The ^{60}Co was placed in a platinum ball fixed by an arm to a ring sutured around the cornea The ball is pressed against and into the base of the tumour The position of both types of applicators are checked by ophthalmoscopy using diathermy marks for the discs and the visible indentation for the ball The isodose curves are different for the two applicators as illustrated in Fig 1

From histological studies of irradiated eyes Moore Stallard & Milner (1931) and Stallard (1955) determined that 3500 rads were lethal to retinoblastoma cells A dose of 3500–4000 rads to the summit of the tumour is recommended (Stallard 1962)

Disc shaped applicators containing the β emitter $^{106}Ru/^{106}Rh$ have been introduced by Lommatzsch (1970) who reported a curative effect on small tumours The limited penetration into the tissue prevented the treatment of tumours with

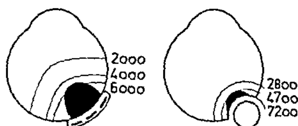


Fig 1

Isodose curves for ^{60}Co applicators Left disc shaped applicator of the Stallard type right ball shaped applicator of the Rosengren type (Amersham England)

a prominence of more than a few millimeters The advantage is the possibility of avoiding radiation complications

External irradiation Martin & Reese (1936) described a technique for treating retinoblastoma by X rays employing special cones and a temporal and nasal port in an effort to deliver an adequate dose to the posterior segment of the eye avoiding the vulnerable anterior segment These radiation damages may be significantly reduced by careful adjustment which unfortunately also spares the anterior retina and vitreous from getting the full therapeutic dose The initially used doses of 8–10 000 R gave rise to severe complications (Reese Merriam & Martin 1949) particularly vitreous haemorrhages and a few patients several years later developed osteosarcoma or rhabdomyosarcoma in the exposed area The currently recommended dose is 3500 R delivered over a three week period (Reese Hyman Tapley & Forest 1949 Reese 1963) which however still gives rise to radiation dermatitis and retardation of cranial growth on the exposed side

Many of the disadvantages of the external radiation were eliminated by the use of megavolt X rays ^{60}Co beams or the linear accelerator whose much more sharply delimited bundle can more easily be directed to bypass the anterior segment of the eye and at the same time the absence of excessive bone absorption and a low skin dose prevent cranial deformities and skin lesions Skeggs & Williams (1966) developed a method with the ^{60}Co beam using a direct anterior field It has the advantage of treating the whole retina and vitreous while the other eye can be effectively shielded but all ocular structures and the brain are irradiated and a cataract inevitably develops within about 18 months (McFaul & Bedford 1970) This latter complication may today be amenable to surgical treatment

Radiomimetic and cytostatic agents have been recommended as a supplement

to radiotherapy Triethylene melamine (TEM) was used by Reese et al (1958) others have employed cyclophosphamide (Stallard 1968) and the more recent drug vincristine sulphate The effect of these toxic agents has recently been doubted (Hopping 1968 Tan & Moller 1973) and they are today used only in the gravest cases

Techniques employed in treatment of present series All patients have been treated solely by irradiation without a supplement of cytostatic agents Light cryo or diathermy coagulations have not been used

Radiation treatment was given with applicators of the Stallard (Amersham England) or Rosengren type The surgical directions given by Stallard (1968) have been followed whereas the Rosengren applicators have been applied only once occasionally twice over a tumour area as it was found difficult to secure the position accurately enough for the several applications recommended by Rosengren & Tengroth (1963) Accordingly only small tumours were treated by the Rosengren applicator

External irradiation was given in early cases by X rays later as ^{60}Co beams Some of the early cases were given doses now known to be inadequate later cases were given ^{60}Co treatments 3–5 per week in a total dose of 4400–6700 rads in 23–79 days The calculated dose in the eye corresponds to a TSD factor of maximally 116–92 and minimally 100–79 (Orton & Ellis 1973)

With the ^{60}Co applicators a minimal tumour dose of 3500–4000 rads was aimed at given with the Stallard discs over a period of approximately 6 days with the Rosengren balls in about 24 hours In using the Stallard discs the doses were calculated from a supposed prominence of the tumour of 7/10 of its base width (Stallard 1968) not taking the actual ophthalmoscopically measured pro minence into account In the later evaluation of these cases it became evident that some of the tumours were rather heavily overdosed

Case histories

It has been the experience of the authors that the most difficult aspect of the radiation treatment is not the surgical localization and fixation of the cobalt disc or ball but rather the interpretation of the postirradiation ophthalmoscopic findings at the follow up examinations For this reason a few cases illustrating the particular problems are reported in detail

No 150315 A boy whose twin brother ultimately had both eyes removed because of retinoblastoma otherwise no family history of retinoblastoma Ophthalmoscopy at the age of 9 months showed nothing abnormal Three months later a tumour was seen above and temporal to the left macula measuring 2–3 PD (papil diameters) with a prominence of 3–4 D

Treatment and course The tumour in the left eye was treated by a Rosengren ball with a calculated dose of 12 000 rads at a depth of 1 mm The tumour regressed and after 6 months appeared as a ring of calcifications (Fig 2) Seven months later a 2 PD tumour was seen in the right eye in the 10 o clock meridian in the peripheral retina This tumour was treated in the same manner as the one in the left eye and disappeared leaving a white atrophic scar Twenty months later (i e 27 months after the first treatment) a new tumour appeared in the left eye in the periphery in the 6 o clock meridian This tumour was also healed by irradiation with a Rosengren ball again giving a dose of 12 000 rads at a depth of 1 mm Ophthalmoscopy under general anaesthesia was performed at regular intervals For more than 4 years (age 6 years) no tumour growths have appeared. The fundus was as illustrated in Fig 3 Visual acuity was 0 3 OD and 0 5 OS

This case shows the multifocal nature of the tumour It is often stated in the literature that multiple tumours are an indication for external irradiation of the entire retina In the eight eyes in the present series with multiple tumours these were most often not simultaneously manifest A ^{60}Co applicator is used for the first tumour which may be cured by the time the second tumour appears In this situation another ^{60}Co disc is preferable

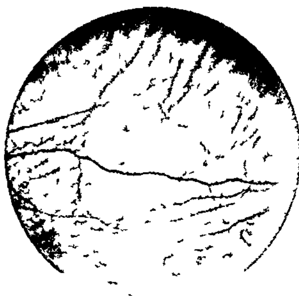


Fig 2
Small ring shaped white scar surrounded by faint retinal oedema Retinoblastoma measuring 2–3 PD with a prominence of 3–4 D treated 6 months earlier by a Rosengren ball Design dose 12 000 rads at a depth of 1 mm (case 180305 left eye)



Fig 3
Right (upper) and left (lower) eye of the same patient as in Fig 2 6 years after ^{60}Co irradiation (case 180368)

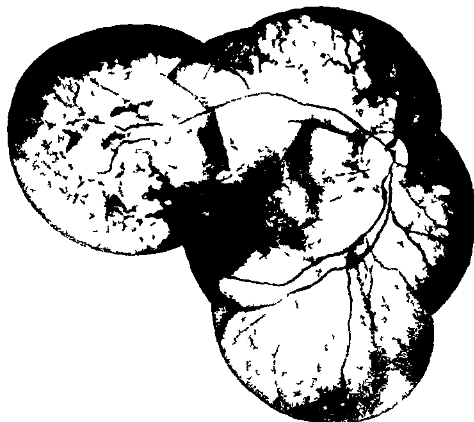


Fig 4
Composite fundusphotograph 1 year after ^{60}Co irradiation (case 290371) Irradiation changes in tumour area Greyish white processes towards the disc

No 290371 A girl with no family history of retinoblastoma At the age of 8 months the mother noticed a yellow reflex in the left pupil and shortly afterwards divergent squint of this eye Ophthalmoscopy of the right eye disclosed a large tumour temporal to the macula measuring 5–6 PD and with a prominence of about 5 D The left fundus was completely filled with tumour most prominent in the temporal half Satellites were seen in the vitreous

Treatment and course The left eye was enucleated as no normal retina was left Microscopy confirmed the diagnosis of retinoblastoma A Stallard ^{60}Co disc (CKA 4) was applied to the right eye until a dose of 4000 rads at a depth of 11.5 mm had been given as recommended by the manufacturer

Regression occurred with flattening marginal pigmentation and a central white fragmented area At examinations 2 and 3 months after radiotherapy the tumour still had a prominence of 3 D The white tumour was surrounded by oedematous retina and pigmentation After 5 and 7 months conditions were unchanged Nine months after the treatment small haemorrhages together with oedema were seen in the tumour area



Fig 5

Composite fundusphotograph same eye as Fig 4 3 months later Progression in the processes towards the disc now showing haemorrhages (case 290371)

Six weeks later conditions were similar except for exudate like areas above and below the macula A further 6 weeks later which was almost 1 year after the initial treatment, the findings were as shown in Fig 4 The areas above and below the macula progressed during the next months (Fig 5) and finally reached the lower disc margin As the nature of the process which now contained some haemorrhages at the disc margin was uncertain and as tumour could not definitely be ruled out it was decided the safest procedure would be to enucleate the eye This was done 1 year and 7 months after the radiation treatment

Examination of the eye Histological examination showed the processes above and below the disc to be subretinal acellular exudates (Fig 6) The retina showed moderate oedema with cystic degeneration and in all layers large swollen hydropic cells with a light and finely granular cytoplasm No inflammatory cell infiltration The tumour area revealed a glial scar with granular calcifications The glial cells were enlarged and hyperchromatic Around the calcifications were remnants of tumour tissue appearing as regular hyperchromatic cells showing in some places rosettes but no proliferation (Fig 7) Oedema and small haemorrhages were present Some of the vessels were thrombosed with swollen endothelium and perivascular fibrosis There was no invasion of tumour in the optic nerve

No 250470 The younger brother of a girl with bilateral retinoblastoma otherwise no family history of retinoblastoma Examined at the age of 4 months In the right eye a 4 PD tumour was seen in and below the macula ending less than 1 PD from the disc. The left eye was normal

Treatment and course A Stallard disc (CKA 10) with an excavation for the optic nerve was placed over the tumour Design dose 4000 rads at a depth of 11.5 mm After 1 month the tumour base appeared greyish and oedematous there were calcifications on the top of the tumour The eye was examined at 1 month intervals and remained unchanged for the next 3 months Six months after irradiation the tumour was described as being chalk white and a delicate veil towards the disc was noted Two months later the disc was oedematous with large newformed vessels The tumour was surrounded by a finely pigmented area and the white calcifications were somewhat diminished Below the tumour and reaching the margin of the disc was an area of greyish oedema with haemorrhages As the other eye was tumour free and as the findings in the right fundus were difficult to interpret an enucleation was decided upon The left eye has been followed regularly and after 9 months still shows no tumour

Examination of the eye In the macula the 2 x 1 mm yellowish remnants of the treated tumour were found The tissue around the disc was whitish and oedematous Microscopic examination in the tumour area showed cicatriceated calcified necrobiotic tumour In

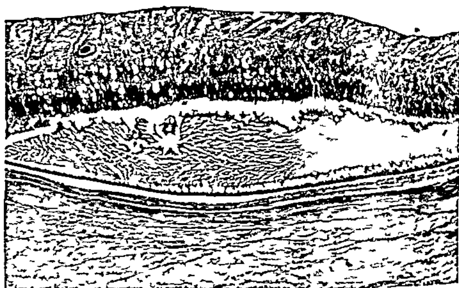


Fig 6

Photomicrograph showing the greyish white process below the disc seen in Fig 5 to be a subretinal acellular exudate The retina is oedematous with swollen hydropic cells *No tumour infiltration Case 290371 magnification × 60*

the internal nuclear layer a considerable hyperplasia was seen in a few places with attempted formation of rosettes The tumour cells were homogeneously hyperchromatic without mitoses and malignant structures The whitish area with haemorrhages around the disc was composed of connective tissue lipid infiltration and oedema but did not contain tumour There was some haemorrhage and fibrosis in the optic nerve sheets but no tumour infiltration

No 0/0561 A boy with no family history of retinoblastoma At the age of 1 month a white yellow pupil was noted On admission at the age of 3 months the left eye was filled by tumour seen immediately behind the lens In the right eye there was a 4 PD tumour situated in the upper nasal quadrant with an 8 D prominence In the 12 o clock meridian another small white tumour with no measurable prominence was seen

Treatment and course The left eye was enucleated Microscopy showed retinoblastoma with rosettes The large tumour in the right eye was treated by a Rosengren ball with a calculated dose of 12 000 rads at a depth of 1 mm Three weeks later the upper smaller tumour was similarly treated the dose being 12 000 rads at a depth of 1 mm After 1 month the large tumour showed a definite regression with chalk white nodules now with only a 4 D prominence The upper smaller tumour had almost disappeared One year later the large tumour consisted of a grey area with a small central whitish scar The prominence was les than 2 D Eighteen months after the radiation treatment a new

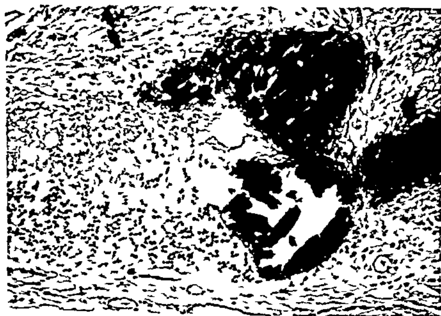


Fig 7

The tumour area of the same eye as shown in Figs 4–6 A glial scar with calcification and remnants of tumour tissue appearing as regular hyperchromatic cells showing in some places rosettes but no proliferation. Magnification x 120



Fig 8
Preretinal cyst appearing after local ^{60}Co irradiation (case 0/0567)

tumour appeared at the 6 30 meridian in the peripheral retina with an estimated size of 1 PD This tumour was treated with a Losengren ball Design dose 12 000 rads at a depth of 1 mm The tumour had disappeared after 1 month During the following month the large central tumour showed no further regression At the site of the upper tumour a large cyst appeared (Fig 8) Later it descended towards the lower tumour where a large scleral necrosis developed A biopsy from this area showed no tumour

Twenty six months after irradiation the large central tumour was seen to be increasing it vaulted over the margin of the disc As recurrence of the tumour was suspected another radiation treatment was given with a crescentic Stallard disc (CKA 19) Design dose 4000 rads at a depth of 7 9 mm After 1 month a vitreous haze had appeared the tumour had definitely grown now extending over the disc There were haemorrhages in the tumour The peripheral irradiated areas were unaltered without signs of recurrence An enucleation was decided upon

Examination of the eye The retina showed atrophic changes gliosis and necrobiotic areas containing histiocytes but no calcification and no surviving tumour cells The findings can be interpreted as being due to irradiation There was no tumour growth in the optic nerve

Radiation effects and complications

Following irradiation the tumour mass changes its appearance becoming a dense white flocculent mass probably due to necrosis and calcification (Stallard 1953)

In the first weeks after irradiation the tumour is surrounded by greyish white oedema Then regression begins and during the next month the chalk white areas make their appearance until the tumour is finally made up of small avascular nodules (Fig 9) Fragments may spread into the vitreous where they appear white refractile and crystalline and should not be mistaken for intra vitreal seeding or recurrence (Haye & Calle 1970) Small tumours (< 6 mm) may disappear totally or leave only small white retino choroidal scars (Fig 2) with larger tumours chalk white slightly prominent debris remains Cysts may develop at the site of irradiation (Stallard 1959) as illustrated in Fig 8 The vessels become attenuated and sclerosed Slight pigmentary disturbances are seen in the surrounding retina and often the choroidal vessels become clearly visible

Complications from irradiation besides cataract glaucoma and keratitis may present as damage to the surrounding retina The fundus becomes light and atrophic in appearance with clearly visible choroidal vessels Ectatic vessels exudation and retinal or vitreous haemorrhages appear The case histories illustrate the late complications encountered with ^{60}Co applicators Small hae morrhages and oedema develop in the tumour and the surrounding retina Exudation progresses and extends towards the optic disc At this stage it was considered that the safest procedure was to enucleate the eye The histological examination showed that the progression was due to exudation and oedema and not to recurrence of the tumour



Fig 9

Irradiated retinoblastoma made up of small chalk white nodules Marked atrophy of surrounding choroid. Large tumour (7 PD × 7 D) treated with Stallard disc CKA 11 *8 months earlier* Design dose 39[illegible]0 rads at a depth of 12 4 mm

Dose calculation with Stallard discs is based on a presumed prominence of the tumour of 7/10 of its base width (Stallard 1968) Retrospectively from the present series it appears that often the tumour protrudes far less resulting in an unnecessarily extensive irradiation and subsequent severe radiation complications It is recommended that the ophthalmoscopically observed prominence be taken into consideration in the dose calculations

Results

The results obtained in the treatment of the present small series are summarized in Table II The Table comprises 28 patients (27 bilateral and 1 unilateral) in whom radiation was considered The figures refer to 55 affected eyes which have been grouped according to the prognostic classification of Reese (1963)

Group I comprises eight eyes of whom six were treated and healed with ^{60}Co applicators Two eyes received an external irradiation treatment now known

Table II
Radiation treatment of retinoblastoma

Prognostic groups	Total no of eyes	Primary enucl no	^{60}Co applicator no	Cure rate %	X ray treatment no	Cure rate*%
I	8	–	6	100	2	–
II	13	–	12	75	1	–
III	4	2	–	–	2	–
IV	6	–	5	20	3	66
V	21	20	–	–	1	–
VI	3	3	–	–	–	–

Prognostic group I considered as very favourable, comprises solitary and multiple tumours less than 4 PD (papil diameters) at or behind equator Group II favourable, comprises solitary and multiple tumours 4–10 PD at or behind equator Group III also considered as favourable comprises any lesion anterior to equator or a solitary lesion greater than 10 PD behind equator Group IV unfavourable comprises multiple tumours greater than 10 PD and any lesion extending anteriorly beyond the limits of ophthalmoscopy Group V very unfavourable comprises massive tumour involving more than half the retina and group VI cases with residual orbital disease optic nerve involvement and extrascleral extension

to be insufficient These two failures therefore cannot be ascribed to the principle of external irradiation The same applies to the single eye in group II treated by X rays Of the remaining 12 eyes in this group 9 were healed (75 %)

In group III two of the four eyes were primarily enucleated (1963 and 1968) because the fellow eye had a smaller tumour Today irradiation of these two eyes would have been attempted before an enucleation was considered Two eyes were treated by X rays with enucleation as the final outcome

Group IV comprises six eyes One out of five treated with ^{60}Co applicators was preserved while two of three receiving X rays were healed Two eyes received both X rays and ^{60}Co discs accounting for the apparent error of 5 + 3 eyes treated out of a group of six

From group V and VI no eyes were saved

In seven cases the tumour was the cause of death of the patient

Discussion

In the literature very favourable results of treatment of retinoblastoma are reported in series treated by ^{60}Co applicators (Stallard 1962 1968 Rosengren 1968 Tengroth & Rosengren 1969) as well as by external beam therapy (Elsworth 1968 Reese et al 1949 Cassady Sagerman Tretler & Ellsworth 1969) or a combination hereof (Bedford Bedotto & MacFaul 1971 Haye & Dollfus 1968 Leunenberger 1968 Lommatzsch 1970) The survey of the present series given in Table II shows that even when a small number of eyes are treated and the experience inevitably less it is possible to save a large fraction of the eyes containing small tumours (groups I and II) As regards the larger tumours (groups III IV and V) the number of treated cases in the present series is so limited that this prevents any conclusions but from the literature it would appear that today it is possible to save a considerable number of these eyes

In the present series most cases were treated by ^{60}Co applicators following the principles of Stallard (1962) or Rosengren & Tengroth (1963) The choice of therapy depends on the localization size and number of tumours Large tumours or three or more tumours in an eye are better treated by external beam therapy (Stallard 1968) Through the large series of Stallard and of Reese and co workers the potential possibilities in ^{60}Co applicators and external irradiation seem to have been established In contrast to this other therapeutic measures e g β irradiation (Lommatzsch 1970) light coagulation (Meyer Schwickerath & Helferich 1968 Hopping 1968 François 1968) cryocoagulation (Lincoff McLean

& Long 1967 Rubin 1968) and diathermy (Weve 1932 Dunphy 195i) seem less well established

The use of cytostatics seems for the present to have receded being recommended only in large tumours in the last remaining eye and in orbital recurrences

Considering the hopeless therapeutic situation that existed before radiation treatment was introduced it may now be concluded that good therapeutic possibilities exist allowing for cure and preservation of usable vision in eyes with small tumours

The therapeutic prerequisite is an early diagnosis Every patient with a white pupil should immediately be referred to an ophthalmologist Careful ophthalmoscopy and photography must be performed usually under general anaesthesia which today incurs only a slight risk It is recommended that all cases of retinoblastoma including the difficult differential diagnostic group be immediately referred to a centre for evaluation and treatment Today radiation treatment can also be recommended in unilateral tumours

The treatment must be followed up by frequent examinations under general anaesthesia at first every 3–4 weeks later with increasing intervals The control must be continued for several years It allows the detection of possible new tumours in the treated eye and in the fellow eye at the same time observing the treated tumours for recurrence These follow up controls should be performed by the therapists who thereby gain the maximum possible experience and also are immediately able to render any additional therapy which might be necessary

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CHRONIC PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA PIGMENTARY RETINOPATHY AND HEART BLOCK (KEARNS SAYRE SYNDROME)

Report of a Case

BY

MARTIN LOWES

Chronic progressive external ophthalmoplegia associated with a pigmentary retinopathy and heart block was described by Kearns & Sayre in 1958 They suggested that the triad represented a syndrome The cases recorded since then are reviewed and summarised and several common features are noted Little attention has previously been paid to the retinopathy A similar case is presented and the pigment changes retinal function and fluorescein angiography are examined There is no clinical evidence for the diagnosis of classical retinitis pigmentosa The prognosis and aetiology of the condition are discussed

Key words chronic progressive external ophthalmoplegia – pigmentary retinopathy – heart block – retinal function – fluorescein angiography – Kearns Sayre syndrome

Kearns & Sayre (1958) presented two cases of chronic progressive external ophthalmoplegia associated with a pigmentary retinopathy and heart block and were of the opinion that the triad could be classed as a syndrome

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The first patient was a 34 year old male of slight build whose main complaint was shortness of breath on exertion The patient exhibited a bilateral ptosis external ophthalmoplegia and a divergent strabismus An electrocardiogram revealed complete heart block Ophthalmoscopic examination showed normal optic discs and retinal vessels The pigment epithelium of the retina had a peculiar stippled appearance and a metallic sheen which was particularly marked around the optic discs where the choroidal vessels were prominent and clearly seen The visual acuity was normal Visual fields revealed a huge enlargement of the blind spots which seemed to match the areas of most markedly disturbed retina around the discs

The second patient was a 17 year old male of small stature who presented with spells of unconsciousness The patient experienced progressive deafness and had to repeat a school grade because of poor work. An electrocardiogram showed complete heart block Bilateral ptosis external ophthalmoplegia and a divergent strabismus were noted Ophthalmoscopy revealed normal optic discs but the retinal arterioles were attenuated The pigment epithelium was mottled and had a fluorescent appearance again most marked around the discs Pigment clumping was observed peripherally Visual acuity was 20/40 in the right eye and 20/60 in the left eye Visual fields showed huge enlargement of the blind spot with breakthrough to the periphery on the right side and an atypical ring scotoma on the left side

The second patient died from a cardiac arrest Histological studies of the retina revealed numerous atrophic areas The pigment epithelium could be identified in only a few places and for the most part was entirely absent In the areas of retinal disturbance only the outer layers of the retina were affected the bipolar and ganglion cell layer were normal and there was no evidence of optic atrophy

Since the original description by Kearns & Sayre a number cases have been recorded in the literature and these are summarised in Table I

It can be seen from the Table that the cases are similar in many respects and the recognition of this triad of symptoms as a syndrome seems justified until the pathomorphological basis has been elucidated The patients can present at any age though commonly in early adulthood (average age on presentation 25 years) The cases are evenly distributed as regards sex A cardiac symptom is the main complaint The patients are frequently of small stature and often of low average intelligence Common to all cases were chronic progressive external ophthalmoplegia cardiac conduction defec s and a pigmentary retinopathy Retinal function has been investigated in only a few cases Associated findings are deafness elevated cerebrospinal fluid protein and abnormal electroencephalographic tracings As far as prognosis is con

Table I

Reported cases of ophthalmoplegia retinopathy and heart block

Cases	1	2	3	4		6	7	8	9	10	11	12	13	14	15	Author's case
Age on presentation (years)	34	17	61	13	35	19	32	39	13	23	16	27	17	14	23	24
Sex	M	M	M	M	M	F	F	F	M	F	F	M	F	M	F	F
Cardiac complaints	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+
Small stature	+	+	O	+	+	O	O	O	+	+	+	+	+	O	+	+
Low average intelligence	O	+	O	—	+	+	O	O	+	—	+	+	—	+	+	+
Ptosis and ophthalmoplegia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac conduction defects	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pigmentary retinopathy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deafness	O	+	O	+	O	O	O	O	+	O	+	+	+	+	+	+
Elevated CSF protein	O	O	—	+	+	O	O	O	+	+	+	+	+	+	O	+
Abnormal ECG	O	O	+	O	+	+	O	O	+	+	+	+	—	+	+	+
Asymptomatic diabetes	O	O	+	—	O	O	O	O	O	—	—	—	+	O	O	+
Enlarged blind spot	+	+	O	—	O	+	+	O	O	+	—	+	O	O	—	+
Abnormal dark adaptation	O	O	+	O	O	O	O	O	O	O	O	O	O	O	O	+
Abnormal ERG	O	O	+	O	O	O	O	O	O	O	+	+	O	O	O	+
Fluorescein angiography	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	+
Dead	—	+	—	+	-	—	—	—	—	+	—	—	—	+	—	—
Cardiac pacemaker	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	+

Key: + present
— absent
O not determined

Cases 1 & 2 Kearns & Sayre (1958) Case 3 Thorson & Bell (1959) Case 4 Jager et al (1960) Case 5 Lind & Frame (1969) Cases 6 7 8 & 9 Kearns (1965) Case 10 Darsee et al (1960) Cases 11 & 12 Drachmann (1968) Case 13 Slaters et al (1971) Case 14 Mattys et al (1973) Case 15 Falling & Nantes (1974)

cerned four patients have died from cardiac causes but the more recent cases have all been successfully treated with a cardiac pacemaker

A similar case is presented and emphasis is laid on the pigmentary retinopathy

Case report

A 24 year old woman previously in good health was admitted to the cardiological department Århus Kommunehospital in March 1974 because of repeated syncopal attacks which had commenced 1 year earlier A complete atrio ventricular heart block was diagnosed and a cardiac pacemaker inserted with good effect Since that time the patient has remained well with no further fainting attacks

The patient was noticed to have a marked ptosis and was referred to the eye department Ptosis of both eyes had begun to develop around the age of 10 years Photographs confirmed that there had been no ptosis in early childhood At the time of admission there was an almost complete external ophthalmoplegia There were no complaints of double vision or night blindness The only other complaint was an incessant tinnitus

Examination revealed a thin woman of small stature (height 153 cm weight 33 5 kg) and of low average intelligence Her physical condition was rather poor which

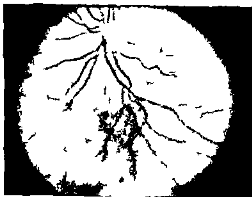


Fig 1

Fundus photograph of the left eye showing marked thinning of the pigment epithelium in the peripapillary area.

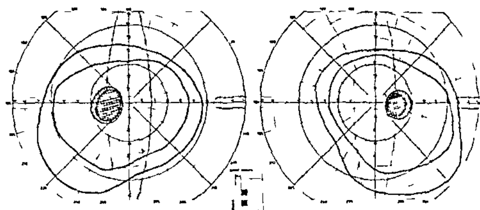


Fig 2
Visual fields showing enlargement of the blind spots

hampered investigations There was a marked bilateral ptosis an almost total external ophthalmoplegia – the patient being able to turn both eyes laterally 5° and less than 5° in all other gaze directions A 5° divergent strabismus was observed There was no convergence but the pupils reacted normally to light

Visual acuity of the right eye was 0 5–1 50 sph and of the left eye 0 5–1 00 sph Colour vision using American Optical pseudoisochromatic plates revealed a mild red/green defect with a medium strong blue/yellow defect

Ophthalmoscopy of both eyes revealed clear media The optic discs appeared normal and there was no attenuation of the retinal vessels There was a marked peripapillary thinning of the pigment epithelium extending about 1½ disc diameters from the margin of the nerve heads The choroidal vessel architecture in this area was also disturbed only the larger choroidal vessels were apparent the choriocapillaris appeared sclerosed (Fig 1) The choroidal vessels in the remainder of the fundus were also clearly visible but appeared normal Diffuse small irregular pigment aggregations exhibiting confluence were seen beginning discretely around the peripapillary atrophic area and extending with increasing density towards the periphery where they were particularly marked The pigmentation in no way resembled the bone corpuscle aggregations seen in retinitis pigmentosa nor was there any pigment sheathing of the retinal vessels The macula areas were covered by pigment and the normal reflexes were absent

Visual fields plotted with both the Bjerrum screen and the Goldmann perimeter revealed marked enlargement of the blind spots but otherwise the fields were normal (Fig 2)

Dark adaptation was measured using the Goldmann Weekers adaptometer and showed a normal cone component but a slightly raised threshold of the rod component Unfortunately the examination could not be carried on for longer than [illegible]0 min because of patient tiredness The curve was biphasic (Fig 3)

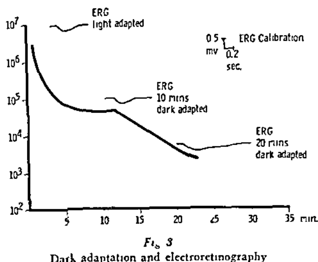


Fig 3
Dark adaptation and electroretinography

Electroretinography exhibited an abnormal response of the negative (–) type. There was an *a* wave of 300 µV with a markedly reduced *b* wave The response in the photopic and scotopic state was unaltered (Fig 3)

Fluorescein angiography in colour revealed normal retinal vessels There was an almost uninhibited view of the choroidal vessel architecture which could be clearly seen at the time of the commencement of the venous filling of the retinal vessels The increased choroidal fluorescence indicates atrophy of the retinal pigment epithelium and on this background pigment accumulations could be seen It was also observed that there was a reduced fluorescence of the peripapillary area suggestive of choroidal vessel atrophy around the optic discs (Fig 4 a) At a later stage the choroidal fluorescence almost obscured the details of the retinal vessels (Fig 4 b)

Biopsy of rectus superior muscle revealed dense fibrous tissue to which a few muscle fibres were attached and as far as it could be observed there were no degenerative changes of the fibre segments or inflammatory reaction Blood vessels were normal no inflammation was present Biopsy of the pectoralis muscle was normal

Otological examination demonstrated a bilateral perceptive hearing loss of 30–35 db most marked for high tones

Neurological examination was normal apart from the ophthalmoplegia and a some what bulbar type of speech Tensilon test was negative Electroencephalography was mildly abnormal with diffuse slow 5–7 cps activity more marked on the left side

Investigations Normal laboratory results included Sedimentation rate 8 mm/hr Haemoglobin 14 g% MCV 89 MCHC 34 Leucocytes 5700 Blood urea 21 mg% Serum creatinine 0 6 mg% SGOT 24 SGPT 18 Serum aldolase 4.3 Serum T_3 4 9% Serum T_4 9 3 ug% PBI 5 4 ug% Serum cholesterol 188 mg% Serum triglyceride





Fig 4a Fig 4b

Fluorescein angiography in colour of the right eye showing increased choroidal fluorescence and pigment accumulations
(a) 18 sec (b) 23 sec

103 mg% (no hypolipoproteinaemia) Serum potassium 3 9 Serum sodium 140 Serum protein 7 3 g % (electrophoretic pattern was normal apart from serum albumin 3 91) VDRL negative GR negative

Abnormal findings included Fasting blood glucose 104–196 mg% (normal 70–110 mg%) LDH 496 (normal 100–400)

Cerebrospinal fluid contained glucose 59 mg% (normal 40–70 mg%) total protein 87 mg% (normal 20–40 mg%) white cells 0/3 red cells 0 3 CSF protein electrophoresis was abnormal – pre albumin 0 8 (< 2) albumin 48 (10–25) α_1 4 1 (< 3) α 8 (< 6) β_1 11 2 (< 6) β 3 5 4 (< 6) γ 9 5 (< 6) No splitting of the γ fraction

Discussion

The case presented here is considered to be a typical example of the Kearns Sayre syndrome The ophthalmoplegia began in early adolescence the patient was well until repeated syncopal attacks due to heart block developed more than 10 years later Cardiac pacemaker treatment was successful (In the past the condition has proved fatal and this emphasises the need for ECG examina

tion in cases of progressive external ophthalmoplegia) The patient was characteristically thin and of low average intelligence There was associated deafness slight dysphonia raised CSF protein and an abnormal EEG Ophthalmoscopy revealed chorioretinal atrophy around the optic discs with marked pigmentary deposits in the fundi there was no optic atrophy and the retinal vessels were normal

Pigmentary retinopathy has been recorded as occurring with progressive external ophthalmoplagia without mention of a heart block There has been some difference of opinion as to the nature of the retinopathy which has been described as varying from a true retinitis pigmentosa to a retinal degeneration with pigmentary changes (McMullen & Hine (1921) Barnard & Scholz (1944) Chamblin & Billet (1950) De Ruyter (1956) Erdbrink (1957) Alfano & Berger (1957)) Koerner (1972) studied the visual sensory aspects in these cases and concluded that the retinopathy was atypical and the visual prognosis was good No mention of ERG was made

As can be seen from Table I little attention has been paid to retinal function Dark adaptation was performed in one patient and ERG in three patients

In case 3 aged 61 years dark adaptation revealed markedly elevated cone and rod thresholds and ERG showed subnormal *a* and *b* waves These findings were consistent with severe damage to cone and rod function In cases 11 and 12 ERG responses were reduced to 50 µV and 300 µV respectively In addition to these cases Kearns (1965) presented two other incomplete cases in which no cardiac abnormalities could be demonstrated The EOG and ERG were both greatly reduced Dark adaptation performed in one patient was almost normal

In the case reported in this paper the retinal function was not severely affected in spite of the marked fundus changes Visual acuity and colour vision were moderatedly disturbed Visual fields revealed grossly enlarged blind spots but the fields were otherwise full Dark adaptation showed a biphasic curve ERG was abnormal but not extinguished Fluorescein angiography demonstrated a severe generalised atrophy of the pigment epithelium with atypical pigment aggregations the retinal vessels were normal and there was no pigment sheathing or optic atrophy

The retinal function studies are incompatible with a diagnosis of true classical retinitis pigmentosa and it is suggested that this term should not be used in describing these patients

As noted by Krill (1967) in a study of rubella retinitis the normal retinal functions in the presence of severe pigmentary changes suggest that the abnormality is one of pigment distribution and not of pigment epithelium cell function However unlike in rubella retinitis the ERG in the present case





Fig 4a Fig 4b
Fluorescein angiography in colour of the right eye showing increased choroidal fluorescence and pigment accumulations
(a) 18 sec (b) 23 sec

103 mg% (no hypolipoproteinaemia) Serum potassium 3 9 Serum sodium 140 Serum protein 7 3 g % (electrophoretic pattern was normal apart from serum albumin 3 51) VDRL negative GR negative

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FOLLOW UP RESULTS OF OCCLUSION AND PLEOPTIC TREATMENT

BY

S R K. MALIK PREM SINGH VIRDI and B K GOEL

Fifty cases of amblyopia including some cases of eccentric fixation, which had previously been successfully treated by various therapies (conventional occlusion. red filter occlusion and pleoptics) were followed up from 12 to 68 months Fifteen cases (30%) deteriorated to pretreatment level during the follow up Deterioration was found to be greater in cases over the age of 15 years and in cases who did not follow the instructions given to them after cessation of therapy

Key words amblyopia – eccentric fixation – occlusion – red filter – pleoptics – amblyopia – therapy

Occlusion therapy and pleoptics are commonly used in the management of amblyopia Though immediate results are encouraging it is necessary to observe the successful cases over long periods owing to deterioration Unfortunately such data are limited Only a few authors have reported the follow up results of the cases successfully treated by pleoptics (Ehrich & Piening 1959 Flynn & Vereckcn 1961 Leydhecker et al 1961 and Tommila & Nordman 1969) and conventional occlusion (Gregersen & Rindziunski 1965 and Fletcher & Silverman 1968)

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Table I
Follow up results

Method of treatment	No of cases	Average age with range	Percentage Maintained	Percentage Partially maintained	Percentage Deteriorated	Follow up period Months
Conventional occlusion (Central fixation)	15	12 years (6–20 years)	46 6	26 7	26 7	12–26 (16)
Conventional eclusion (Eccentric fixation)	12	14 years (5–22 years)	50 0	16 6	33 3	12–30 (19)
Red filter occlusion	15	14 years (6–35 years)	33 3	33 3	33 3	12–19 (21)
Pleoptics (Cupper s technique)	8	14 years (6–22 years)	62 5	12 5	25 0	27–68 (36 2)
Total	50	13 4 years (5–35 years)	46 0	24 0	30 0	21 4 12–68 months

Maintained Vision and fixation remained unaltered during the follow up
Partially maintained – Vision and fixation deteriorated but did not fall to the pretreatment levels
Deterioration – Vision and fixation deteriorated to the pretreatment levels

Material and Methods

A total of 256 cases of amblyopia with and without eccentric fixation were treated by various means (conventional occlusion non conventional occlusion red filter occlusion and Cuppers technique) Of these 143 patients showed improvement but only 50 could be followed for a period longer than 1 year These included 15 cases of central fixation and 12 cases of eccentric fixation *treated by conventional occlusion 15 cases treated by red filter* and 8 cases by pleoptics The visual acuity refractive error and state of binocular vision were determined before and after treatment

The age of onset of the amblyopia was not incorporated into the study owing to the difficulty in obtaining exact information from the patients

Results

The average age of the patients in this series was 13.4 years (ranging from *5 to 35 years) There were two cases over 30 years one aged 31* and the other 35 years The period of follow up ranged from 12 months to 68 months The result at reexamination was recorded as maintained if both vision and fixation had remained unaltered during the follow up period partially maintained if vision and fixation had deteriorated but not to the pre treatment

Table II

Relationship between the age of patients and deterioration

Age of patient	*No of cases*	*Deteriorated*	*Percentage deterioration*
0 – 5 years	3	–	0
6 – 10 years	16	2	12.5
11 – 15 years	[illegible]	4	50.0
Above 15 years	[illegible]	9	52.9
Total	50	15	30.0

Deterioration was greater in patients *over the age of 15 years* This difference was statistically significant ($\chi^2 = 6.2$ $P < 0.05$)

Table III
Relationship between carrying out of instructions and deterioration

Instructions carried out	No of cases	Deteriorated	Percentage deterioration
Yes	42	9	21 4
No	8	6	75 0

The rate of deterioration in the two groups was found to be statistically significant ($\chi^2 = 6\ 81$ $P < 0\ 05$)

level and deteriorated if vision and fixation had fallen to the pre treatment level (Table I) It was observed that the rate of deterioration was the lowest in cases treated by pleoptics (25 %) followed by cases of central fixation treated by conventional occlusion (26 7 %) However it was the same (33.3 %) in cases of amblyopia with eccentric fixation treated by conventional occlusion and by red filter The percentage of maintenance varied from 62 5 % in cases treated by pleoptics to 33 3 % in cases of eccentric fixation treated with red filter

No relationship was observed between the percentage of deterioration and the visual acuity achieved immediately after the treatment the degree and state of refractive error and the presence or absence of binocular vision

The percentage of deterioration was higher (52 9 %) in cases over the age of 15 Patients in the age group up to 10 years showed deterioration in 12 5 % and those between 11 and 15 years in 25 % of the cases (Table II)

The rate of deterioration was significantly lower in cases who followed the instructions given to them at the completion of therapy (Table III)

Discussion

It is of interest to note that on the average 30 0 % of the improved cases reverted to the original level of amblyopia and that there was no difference in the maintenance of improvement in cases which were treated differently It appears that in most of these cases we have not been able to eliminate the basic inhibitory influences which slowly undo the changes brought about by therapy This is further supported by our observation that the deterioration

rate was much less in those cases which came for regular check up and followed instructions A conventional occlusion intermittent occlusion or reduction of the vision of the better eye by one or two lines helps to reduce the rate of deterioration In cases showing a repeated tendency to deteriorate prolonged partial occlusion of the good eye (using clear nail varnish) was found to be most helpful The results of follow up cases treated by pleoptics as reported by Aust (1962) and Aichmair & Frey (1968) are in general agreement with those obtained in the present series Some authors (Flynn & Vereecken 1967) have reported better maintenance while others (Ehrich & Piening 1959 Girard et al 1962 Von Noorden & Lopsius 1964 Leydhecker et al 1967 and Tommilla & Nordman 1969) have observed poor maintenance of the improvement

The rate of deterioration in cases of amblyopia with central and eccentric fixation treated by conventional occlusion was 26 7 % and 33 3 % respectively This is much higher than that reported by Gregersen & Rindziunski (1965) These authors treated their cases at the age of 4 to 5 years and followed them up until the age of 17 years They found deterioration in 16 9 % of the cases 58 6 % partially maintained and 24 5 % fully maintained the improvement The full maintainance of visual gain was however much better in our series 46 6 % and 50 0 % (Table I) This difference in results appears largely due to the type of case in the two series the age at which the treatment was started and the length of the follow up period Fletcher & Silverman found that 40 0 % of the cases maintained the improvement after 1 year of successful treatment However 23 of their 69 cases did not fully improve and showed a persistent amblyopia The authors commenced treatment of their cases before the age of 5 years This persistent amblyopia was not related to co operation of the patient anisometropia or age at which the treatment was given In our experience co operation of the patient is the single most important factor We did not find any significant relationship between the age of the patient and the immediate success of the therapy barring those in whom this was started before the age of 5 years Fletcher & Silverman (1968) observed that the patients with binocular vision were more prone to have persistent amblyopia In this series there was no difference between the group with and the group without binocular vision Gregersen & Rindziunski (1965) felt that a good position of the eyes and normal binocular vision leads to stabilization of the improvement They suggested a follow up period until the age of 20 years

Analysis of various factors which influence the maintenance of improvement showed that the level of final visual acuity state of binocular vision degree and type of refractive error and degree of anisometropia were insigni

ficant However the maintenance was found to be better in cases below the age of 15 years and those who followed the instructions given after the completion of the amblyopia therapy Follow up is important to counter any subsequent deterioration of vision

It would have been interesting to study the relation between the age of onset of amblyopia and the success rates but unfortunately it has proved most difficult to obtain the relevant information from the patients Patients with anisometropic or ametropic amblyopia first became aware of the anomaly at a routine medical examination which in the majority of our cases was performed rather late in life

Acknowledgement

We are grateful to Mr Baljit Singh Orthoptist Department of Ophthalmology Maulana Azad Medical College and associated Irwin & G B Pant Hospitals New Delhi for the technical assistance provided in this study

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REMARKS ON THE AQUEOUS HUMOR/PLASMA RATIOS FOR AMINO ACIDS AND RELATED COMPOUNDS IN PATIENTS WITH VARIOUS CHRONIC OCULAR DISORDERS

BY

F SCHØNHEYDER N EHLERS and B HUST

The concentration of 19 amino acids and related compounds was simultaneously determined by means of ion exchange chromatography in the aqueous humour (C_{aq}) and blood plasma (C_{pl}) of 40 patients with various chronic ocular disorders The physiological aspects will be published elsewhere It is of interest to note that very few significant differences have been found between the C_{aq}/C_{pl} ratios for the groups into which the material could be clinically divided This enables the conclusion to be drawn that determination of C_{aq}/C_{pl} for amino acids and related compounds are not of diagnostic importance in the chronic ocular disorders which were studied

Key words aqueous – blood plasma – amino acids

It is widely accepted that amino acids and related compounds pass from the blood to the aqueous humour mainly through the ciliary epithelium while other routes are of less importance The data reported in the present study relating to 40 patients with various chronic ocular disorders suggest that in man most of the amino acids are transported into the aqueous humour by a process of secretion However no evidence for secretion has been found concerning the transport of taurine urea glutamic acid glycine and cystine but neither has it been excluded (Schonheyder Ehlers & Hust 1976)

Table I
Examined cases

No	Sex	Date of birth	Date of invest	Clinical diagnosis	Anaesthesia
1	M	26 02 02	04 10 72	Cortical cataract	LA
2	M	02 01 24	05 10 72	Cortical cataract	LA
3	M	02 07 94	10 10 72	Cortical cataract	LA
4	M	17 01 84	23 10 72	Cortical cataract capsular glaucoma	LA
5	F	05 11 03	26 10 72	Total cataract	LA
6	M	07 01 87	06 11 72	Aphakia	LA
7	M	19 05 72	07 11 72	Congenital cataract (total)	GA
8	F	18 09 12	07 11 72	Total cataract	LA
9	F	09 08 10	13 11 72	Angle closure glaucoma	LA
10	F	26 01 05	14 11 72	Cortical cataract	LA
11	F	25 11 03	21 11 72	Total cataract	LA
12	M	20 03 69	28 11 72	Congenital cataract (total)	GA
13	F	20 07 65	12 12 72	Congenital cataract (zonular)	GA
14	F	12 04 16	19 12 72	Cortical cataract	LA
15	F	01 01 32	10 01 73	Cortical cataract	LA
16	F	26 12 34	10 01 73	Total cataract	LA
17	M	26 07 11	11 01 73	Total cataract	LA
18	M	31 10 04	18 01 73	Total cataract	LA
19	M	03 07 96	22 01 73	Choroidal melanoma	GA
20	F	23 10 17	23 01 73	Cortical cataract	LA
21	M	12 12 00	29 01 73	Total cataract	LA
22	M	27 08 99	30 01 73	Total cataract	LA
23	M	02 02 92	06 02 73	Cortical cataract	LA
24	M	1[illegible] 03 90	06 02 73	Cortical and nuclear cataract	LA

It is the purpose of the present paper to give more detailed information about the patient material which forms the basis for these studies

Dickinson Durham & Hamilton (1968) have compared the amino acid concentrations in the aqueous humour in one normal person and a group of cataract cases In addition they compared the aqueous humour to plasma ratios (C_{aq}/C_{pl}) for each amino acid in one normal person the average of three cataract cases one case of secondary aqueous and one case of malignant melanoma As regards the latter series it is stated that Where the ratios were markedly different from the normal case it was always due to higher or lower concentrations in the aqueous fluid

Table I (cont)
Examined cases

No	Sex	Date of birth	Date of invest	Clinical diagnosis	Anaesthesia
25	F	*23 02 16*	*13 02 73*	Nuclear cataract	LA
26	F	03 09 08	14 02 73	Total cataract	LA
27	F	18 10 13	15 02 73	Cortical cataract	LA
28	F	09 06 84	27 02 73	Total cataract	LA
29	F	03 11 05	06 03 73	Cortical cataract	LA
30	M	18 07 67	08 03 73	Lens luxation	GA
31	M	23 03 00	05 03 73	Cortical cataract glaucoma simplex	LA
32	M	14 12 64	23 03 73	Lens luxation	GA
33	M	06 06 72	28 03 73	Hyperglycinemia)	GA
34	M	20 10 43	24 04 73	Malignant melanoma	GA
35	F	10 05 18	28 05 73	Retinal medulloepithelioma and detachment	LA
36	F	14 01 71	17 05 72	Congenital cataract homocystinuria	GA
37	F	14 01 71	30 10 72	Congenital cataract homocystinuria	GA
38	F	14 01 71	22 07 73	Congenital cataract homocystinuria	GA
39	M	13 12 26	07 09 73	Total cataract keratoconus Downs syndrome	GA
40	M	05 05 73	12 09 73	Congenital cataract (nuclear)	GA
41	M	02 02 97	19 09 73	Normal eye maxillary carcinoma	GA
42	F	03 08 28	12 09 73	Cortical cataract Downs syndrome	GA
43	F	27 11 12	14 09 73	Malignant melanoma	GA

GA = General anaesthesia
LA = *local anaesthesia lidocain 20 mg/ml + adrenalin 5 μg/ml*
) the case will later be published by N J Brandt et al

The same year Curtius Martenet & Anders (1968) published a study of the concentrations of free amino acids in humour in homocystinuria and normal controls

Material and Methods

The material comprises 43 samples of aqueous humour and simultaneously obtained blood plasma from 40 patients (Table I) All patients except one were in patients at the Department of Ophthalmology Århus Kommunehospital One set of samples (no 33) was from a patient at the Department of Ophthalmology Københavns Kom

munehospital The patients were fasted for about 15 hours before the samples of aqueous and blood plasma were taken Most patients had retrobulbar Lidocain adrenaline anaesthesia while the remainder were given a general anaesthetic (Fluothane) Patients with cataract or with lens luxation had pupils dilated by topical atropine on the day of operation

Approximately 100 μl of aqueous humour was withdrawn through a 0 6 mm canula inserted under macroscopic control through a keratotomy The amount of aspirate was determined by weight and then diluted with citrate buffer (pH 2 2) to a suitable volume A sample of blood was simultaneously taken from an arm vein into heparinized glasses and immediately centrifuged The plasma formed was deproteinized with picric acid and prepared for chromatography using the technique described by Stein & Moore (1954)

Quantitative chromatographic analysis was carried out with a Beckman's Amino Acid Analyzer 120 C following the Instruction Manual issued by the Spinco Division of Beckman Instruments Inc To calculate the free amino acid concentration in mmoles/kg H_2O the water contents of aqueous and of plasma were taken as 99 % and 91 % respectively of their total weight For each set of samples the ratio C_{aq}/C_{pl} was calculated The somewhat voluminous lists with the individual determinations of C_{aq} and C_{pl} can be obtained on application to the authors

The material has been divided in four groups of approximately equal size This was not quite so simple because the material comprised both adults and children, and moreover some of the patients suffered from more than one chronic disorder The 13 adults placed in group (1) are characterized by having an incipient cortical cataract but the group is not absolutely homogenous as case no 4 had in addition a capsular glaucoma case no 24 had nuclear opacification case no 31 simple glaucoma and case no 42 Downs syndrome Eleven adults in group (2) had mature, total cataract In this group case no 39 had also keratoconus and Downs syndrome The groups (1) and (2) were formed in an attempt to elucidate a possible link between amino acids and cataractogenesis Group (4) is composed of nine children with congenital disorders (Table I nos 7 12 13 36 37 38 and 40 with cataract and nos 30 and 32 with lens luxation) The cases 7 12 13 30 32 36 37 and 38 as well as no 33 with hyperglycinaemia have previously been presented (Ehlers & Schønheyder 1974) The remaining group (3) of nine patients is particularly heterogenous, comprising aphakia (case no 6) angle closure glaucoma (case no 9) isolated nuclear cataract (case no 25) a normal eye (no 41) from a patient with a maxillary carcinoma and four cases of intraocular malignancy (nos 19 34 35 and 43) In addition to these eight adults a child with congenital hyperglycinaemia (no 33) was included In this child no eye disease could be demonstrated but it was considered of interest to determine the C_{aq}/C_{pl} in a case of increased C_{pl} for glycine

Statistical evaluation of the data was made by computer following the methods of Sokal & Rohlf (1969) For some of the most unevenly distributed groups of data the Kolmogorov Smirnov test was performed and no significant deviation from Gaussian distribution was revealed Differences between the x of the four groups were evaluated by the SNK procedure

Table II

C_q/C_{pl} ratios for amino acids and related compounds

	Incipient cortical cataract			Mature total cataract			Other eye diagnosis			Congenital eye disorders		
	N	x_1	s e m	N	x_2	s e m	N	x_3	s e m	N	x_4	s e m
Taurine	13	1.20	0.139	11	1.04	0.078	9	0.71	0.073	9	0.76	0.092
Urea	13	0.98	0.047	11	0.85	0.041	9	0.88	0.056	9	0.81	0.044
Threonine	13	1.16	0.070	11	1.19	0.070	9	1 0	0.094	9	1.24	0.081
Serine + Glutamine	13	1.29	0.074	11	1.28	0.070	9	1.38	0.088	9	1.14	0.055
Glutamic acid	13	0.16	0.0194	11	0.18	0.040	9	0.20	0.051	9	0.33	0.064
Proline	13	0.19	0.076	10	0.18	0.010	9	0.24	0.048	7	0.26	0.044
Glycine	13	0.12	0.018	11	0.10	0.012	9	0.16	0.046	9	0.18	0.044
Alanine	12	0.97	0.067	11	0.96	0.053	9	1.00	0.091	9	1.06	0.096
½ Cystine	12	0.11	0.0095	10	0.09	0.0090	7	0.13	0.047	–	–	–
α ANBA	12	1.37	0.133	11	1.57	0.141	8	1.39	0.191	7	1.14	0.102
Valine	13	1.27	0.0540	11	1.39	0.062	9	1.43	0.123	9	1.34	0.059
Methionine	13	2.54	0.174	11	2.54	0.157	9	3.00	0.200	9	2.26	0.184
Isoleucine	13	1.23	0.069	11	1.40	0.109	9	1.34	0.118	9	1.24	0.128
Leucine	13	1.35	0.075	11	1.49	0.084	9	1.48	0.139	9	1.25	0.098
Tyrosine	13	1.82	0.101	11	1.81	0.081	9	1.92	0.192	9	1.86	0.124
Phenylalanine	13	1.97	0.093	11	2.02	0.077	9	2.18	0.194	9	1.96	0.193
Lysine	13	0.65	0.038	9	0.58	0.033	6	0.765	0.065	5	0.77	0.103
Histidine	13	0.88	0.041	8	0.96	0.105	6	0.95	0.083	5	0.92	0.041
Arginine	13	1.57	0.140	8	1.39	0.097	6	1.57	0.054	5	1.67	0.160

The underlinings indicate maximum nonsignificant ranges (Sokal & Rohlf 1969) ($P < 0.05$)

Table III
Aqueous concentrations of amino acids

	Incipient cortical cataract			Mature total cataract			Other eye diagnosis			Congenital eye disorders		
	N	x_1	s.e.m.	N	x	s.e.m.	N	x_3	s.e.m.	N	$\bar{x}_4$	s.e.m.
Taurine	13	0.091	0.010	11	0.063	0.0039	9	0.043	0.0053	9	0.037	0.0031
Urea	13	5.65	0.83	11	5.21	0.35	9	1.80	0.422	9	5.00	0.93
Threonine	13	0.119	0.0072	11	0.129	0.0094	9	0.167	0.018	9	0.118	0.011
Serine + Glutamine	13	0.840	0.078	11	0.830	0.046	9	0.879	0.049	9	0.745	0.032
Glutamic acid	13	0.008	0.00067	11	0.008	0.0012	9	0.013	0.0025	9	0.014	0.0011
Proline	13	0.047	0.0058	10	0.042	0.0041	9	0.0051	0.011	7	0.050	0.0072
Glycine	13	0.026	0.0028	11	0.024	0.0042	9	0.061	0.021	9	0.048	0.014
Alanine	12	0.330	0.018	11	0.313	0.011	9	0.371	0.072	9	0.256	0.022
½ Cystine	12	0.015	0.0014	10	0.012	0.0012	7	0.020	0.0094	–	–	–
α ANBA	12	0.024	0.0023	11	0.030	0.0033	8	0.034	0.0039	7	0.035	0.0053
Valine	13	0.344	0.011	11	0.315	0.021	9	0.294	0.023	9	0.305	0.034
Methionine	13	0.043	0.0016	11	0.045	0.0033	9	0.050	0.0040	9	0.110	0.053
Isoleucine	13	0.057	0.0029	11	0.069	0.0054	9	0.064	0.0053	9	0.076	0.0090
Leucine	13	0.121	0.0047	11	0.150	0.0096	9	0.141	0.010	9	0.150	0.070
Tyrosine	13	0.093	0.0047	11	0.101	0.0060	9	0.095	0.010	9	0.099	0.019
Phenylalanine	13	0.092	0.0036	11	0.097	0.0039	9	0.091	0.0077	9	0.107	0.0067
Lysine	13	0.158	0.0061	8	0.160	0.012	6	0.193	0.022	5	0.169	0.033
Histidine	13	0.069	0.0039	8	0.068	0.0049	6	0.069	0.0049	5	0.070	0.0041
Arginine	13	0.106	0.0064	8	0.106	0.0039	6	0.104	0.011	5	0.101	0.0085

The underlinings indicate maximum nonsignificant ranges (Sokal & Rohlf 1969). For taurine $P < 0.01$.
For threonine, glutamic acid and alanine $P < 0.05$

Results

Table II summarizes the average C_{aq}/C_{pl} ratios for the 19 compounds studied and is divided into the four groups described in the preceding section. No differences between the $\bar{x}$ values significant at the 1 % level were found for any of the 19 compounds At the 5 % level differences were found for taurine glutamic acid and methionine For taurine $\bar{x}_1$ was higher than $\bar{x}$ while $\bar{x}_3$ and $\bar{x}_4$ were lower than $\bar{x}$ The difference between $\bar{x}_3$ and $\bar{x}_4$ was not significant For glutamic acid $\bar{x}_4$ was higher than the other $\bar{x}$ values which did not differ from each other For methionine $\bar{x}_3$ was higher than $\bar{x}_1$ and $\bar{x}$ and $\bar{x}_4$ lower than $\bar{x}_1$ and $\bar{x}$

Table III shows the average C_{aq} values for the same four groups When subjected to the same statistical test as Table II differences were found at the 1 % level for taurine alone $\bar{x}_1$ was higher than $\bar{x}$ $\bar{x}_3$ and $\bar{x}_4$ were lower than $\bar{x}$ $\bar{x}_3$ and $\bar{x}_4$ were different only at the 5 % level

At the 5 % level differences were also found for threonine glutamic acid and alanine but not for methionine For threonine $\bar{x}_3$ was higher than the other $\bar{x}$ values for glutamic acid $\bar{x}_1$ and $\bar{x}$ were lower than $\bar{x}_3$ and $\bar{x}_4$ For alanine $\bar{x}_1$ was higher than $\bar{x}$ and $\bar{x}_3$ and $\bar{x}_4$ lower than $\bar{x}$ and $\bar{x}_3$ which did not differ

For taurine and glutamic acid the significant changes for C_q and C_{ap}/C_{pl} occur between almost the same groups (Tables II and III) and thus C_{aq} for these substances contributes towards the understanding of the variation of C_{aq}/C_{pl}

Discussion

No definitely significant ($P < 0\,01$) and only a few probably significant ($P < 0\,05$) differences were found between the average C_{aq}/C_{pl} ratios of the four groups We conclude from this that no evident alterations in the transport of the amino acids and related compounds are probable for the disorders included in the material and we therefore find it justified to consider the material as homogenous with respect to transport of the studied substances This assumption was a prerequisite for the physiological part of this study published elsewhere However the same conclusion means that in the chronic eye diseases which were studied no diagnostic or pathogenetic information can be expected from the determination of C_q/C_{pl} ratios It can especially be noted that even the most homogeneous groups (1) and (2) with incipient and total cataract respectively have not shown any difference so that the examination

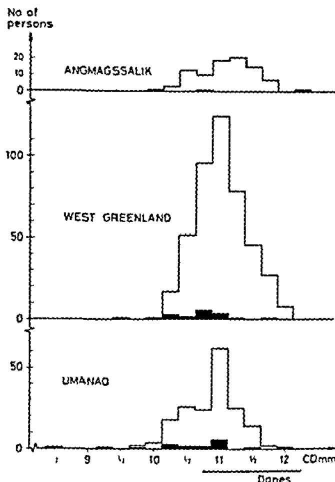


Fig 1

Corneal diameter (CD) distribution in 722 female Eskimos over 40 years Umanaq the four other West Greenland groups and Angmagssalik are shown separately Shaded parts of the histograms indicate the number of a c g patients within the samples Median (†) and range for 33 Danes are shown at the bottom.

CD-ACD correlation

The association between the frontal and sagittal dimensions – CD and ACD – of the anterior chamber was analysed in the 263 ♂ and 298 ♀ Umanaq Eskimos whose right eyes were measured The influence of age on ACD was eliminated using the deviation score (DS) recently described (Alsbirk 1975 b) In this way the age independent coefficients of correlation between CD and

Table III

Distribution of CD in 38 a c g patients and in their background population
Prevalence of a.c.g at each CD level is shown for females (in per cent)

CD class marks (mm)	♂		♀		
	A c g patients no	Population no	A c g no	Patients per cent of class	Population no
12¹/₄					1
12		11		2 %	9
11³/₄	1	18	1		36
11¹/₂	2	35	1	1 %	75
11¹/₄	1	55	1	1 %	125
11		77	10	5 %	206
10³/₄	1	27	9	7 %	130
10¹/₂		15	4	4 %	91
10¹/₄		8	6	16 %	38
10		6			6
9³/₄			1		2
9¹/₂		1		9 %	1
9¹/₄					1
8¹/					1
Total no	5	20 % 253	33	46 %	722
Calculated median	11 44	11 10	10 78 mm		10 99

ACD were found to be r = +0.52 in males and r = +0 45 in females (r = + 0 47 as a pooled estimate) i e the covariation was highly significant ($P \ll 0\,01$)

Family correlation of CD

In the Umanaq CD survey population the total number of unrelated marital pairs with child(ren) examined was analysed (Table IV and Fig 2) With respect to corneal size the adult male and female children resembled their parents to much the same fairly high degree (and consequently they were pooled) On the other hand no husband wife correlation was found Thus a considerable genetic influence was suggested

Table IV
Family resemblance of corneal diameter in general population families

Relationship (y) (x)	No of pairs	b_{yx}	s_b	r	r_s	P
Child on midparent	60	0 80	0 14	0 56	0.54	$P < 0\,001$
Husband on wife	43	0 01	0 16	0 01	0 03	n.s

Regression coefficient b_{yx} standard error of b_{yx} s_b correlation coefficient r
Spearman's rank correlation coefficient r_s

Microcornea

The population study revealed six persons with a pronounced asymmetric microcorneal appearance The smaller eyes ranged from 9–10½ mm transversally and 8–9½ mm vertically while the fellow eyes on an average measured 10–11 mm The microcorneal eyes had shallow chambers ACD 1 4 1 4 1 7 1 7 2 0 and 2 4 mm 0 ?-0 ? mm below the fellow eyes but no a c g was diagnosed The cases amounted to 0 3 % (1/358) of the male and 0 6 % (5/853) of the female population A total of 1 9 % (32/1 656) of female eyes and 1 3 % (9/709) of male eyes had CD values ≤ 10 mm and as a rule a high degree of symmetry was found also in this group

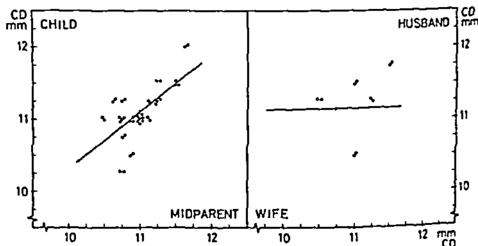


Fig 2
Child on midparent versus husband on wife regressions and scattergrams of corneal *diameter (CD) A significant regression is found in left graph* (b = 0 8)

Discussion

The high prevalence of primary angle closure glaucoma (a c g) in Eskimos motivated this CD survey Furthermore the study was intended to contribute to the anthropometric and genetic knowledge of corneal size.

The *limited precision* of the measurements was a problem which complicated the study However the poorly demarcated corneal border virtually prevents any great improvement in precision The simple procedure used made it possible to examine large population groups Non Gaussian distributions were obtained probably due in part to the broad classes inherent in the method The study of Peters (1924) showed a Gaussian distribution (in 512 children aged 5–16 years) while Ortlepp s (1966) control group – as my material – was skewed towards lower values However none of the comparisons in the study were made with tests which assume normality In Table II medians as well as means are given Generally only small discrepancies are seen and the s d values correspond to those of other studies

The *variation of CD* with *age* and *sex* closely agreed with that of European populations only a very small decrease with age but a pronounced sex dif ference ($\overline{\Delta}$ 0 2 mm) was found in large groups (cf Priestley Smith 1890 Johansen 1947)

For the purpose of ethnic comparison the *group of Danes* in Umanaq was included here as well as in the ACD survey This also helped to check the method The sample differed conspicuously from the Eskimos and agreed with other Caucasian samples (cf Introduction)

Kaiser (1976) demonstrated that the corneal size was fully developed early on in preschool age Friede (1933 in Germany) measured 10 940 eyes using Wessely s kerato meter transversally and vertically mean value 11.56 mm Johansen (1947) measured 159 adult Danes (after death) and found the following mean values 11 77 mm (♂) and 11 60 mm (♀) transversally 11 41 mm (♂) and 11 15 mm (♀) vertically Fledelius (1975) examined 237 Danish 10 year old children using a Wessely keratometer trans versally and obtained the following results (means (and s d.)) ♂ 11 63 mm (0 43) and ♀ 11 46 mm (0 46)

Thus the *ethnic difference* in corneal size between Eskimos and Caucasians appears to be an established fact. On the basis of this the *geographical variation* in Greenland Eskimos seems to be rather small and unimportant although a statistically significant variation was found (Table II) The Umanaq groups of males and females showed the lowest values and those of Angmagssalik the highest *A priori* this result was unexpected because the East Greenlanders represent the purest group of Eskimos in Greenland A partly corresponding pattern was found in the ACD survey (Alsbirk 1974 b) in which the Ang

magssalik group was intermediate Anthropological aspects of these findings will be discussed elsewhere

An important epidemiologic aspect was *the possible association between prevalence rate of a c g and the level of CD* The large elderly population groups of the survey formed a most suitable material for a study of this problem as for the recent epidemiologic study of a c g and chamber depth (Alsbirk 1975 a) An association was found but it turned out to be much less pronounced than in the case of a c g and ACD Unfortunately the discriminative value of the CD measurement in a population like this seems to be small as more than half of the elderly female population had CD values which suggested a relatively high risk of a c g (Table III)

A fairly clear pattern seems to emerge from this population study – as from that of the correlated parameter ACD Eskimo a c g patients have the same CD distribution as Caucasian a c g patients On the other hand the corresponding background populations differ considerably as the Eskimos show mean values about 0 5 mm below those of Caucasians near the general mean of a c g patients

The *family study* revealed a high degree of resemblance between children and parents suggesting a heritability estimate of h = 0 8 judged by the child on midparent regression (Table IV cf Alsbirk 1975 b) Thus the *variation of CD seems to be mainly genetically determined* although some reservation is called for due to the crude non Gaussian distributions A similar familial regression study has not been published before Peters (1924) selected 10 families through microcorneal probands and 11 families through macro corneal probands The attempt to establish a dominant or recessive mode of inheritance failed but a high degree of family resemblance seemed to exist in both groups Fskelund (1938) reported the corneal width of 42 children and their 14 pairs of parents A regression analysis of these data revealed a child on midparent estimate corresponding to my result ($b_{yx} \pm s_b = 0\,59 \pm 0\,13$) Tomlinson & Leighton (1973) showed that corneal diameter and height in sibs and children of a c g patients were significantly smaller than in controls

In conclusion the study showed that Greenland Eskimos are relatively microcorneal compared with Caucasians The small cornea characteristic of Eskimos seems to be one of the reasons for their predisposition to primary angle closure glaucoma (a c g) The high degree of family resemblance observed suggests that the size of cornea is to a large extent genetically determined

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Case Report

(Aa J 261021) A 52 year old man presented to an eye doctor with complaints due to presbyopia As long as he could remember there had been a black spot in front of the left eye to which he had become quite accustomed and he had never consulted an eye doctor concerning this There was no previous history of eye disease nor was there any family history of retinoblastoma The patient had three children aged from between 14 to 25 years all of whom had normal ocular fundi none of them had any children

Visual acuity was normal in both eyes The right fundus was normal but in the left eye ophthalmoscopy revealed a vertically oval atrophic area approximately 4 X 6 disc diameters in size situated two disc diameters above the optic disc (Fig 1) This area was crossed by several choroidal and a few retinal vessels There was some pigmentation around the periphery of the lesion and from the centre arose a white chalk like lobulated tumour prominating 5–6 dioptres and containing a thin vessel loop on its nasal edge Small exfoliated particles were seen floating in the corpus vitreum There was a partial corpus detachment in the upper and temporal regions with small glistening white flakes on its posterior surface The eye did not show any sign of inflammation Radiological examination using plain X ray studies revealed a small irregular central shadow which could represent calcification in a tumour

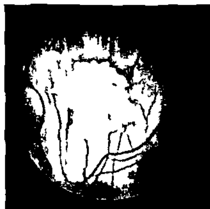


Fig 1
Left fundus with spontaneously regressed retinoblastoma



Fig 2
Fundus with regression of retinoblastoma after radiation treatment
(Ehlers N & Kaae S)

Discussion

The diagnosis of spontaneous regression of retinoblastoma has in all the published cases been based on one or more of the following three criteria often in connection with a family history

(1) A characteristic fundus picture

(2) A clinical diagnosis of bilateral retinoblastoma with removal of the most affected eye and in most cases microscopic verification followed by regression in the fellow eye

(3) The presence of calcified tumour cells in a removed phthisical eye

Table I
Ocular dimensions pressures and rigidity

		Glaucoma simplex	Ocular hypertension	Fibrillopathia epitheliocaps	Total
Corneal thickness	R	521 ± 003	542 ± 007	.539 ± 007	.536
	L	526 ± 003	557 ± 007	538 ± 007	543
Depth of anterior chamber	R	2 61 ± 147	2 97 ± 071	2 80 ± 085	2 82
	L	2 72 ± 130	2 82 ± 081	2 67 ± 099	2 74
Lens thickness	R	4 74 ± 113	4 38 ± 067	4 67 ± 096	4.58
	L	4 72 ± 132	4 49 ± 056	4 92 ± 107	4 71
Length of vitreous	R	14 93 ± 238	14 71 ± 134	15 03 ± 156	14 89
	L	14 79 ± 221	14 75 ± 126	14 99 ± 169	14 85
Axial length	R	22 29 ± 223	22 05 ± 160	22 50 ± 189	22 28
	L	22 23 ± 212	22 04 ± 142	22 58 ± 204	22 29
Applanation tension	R	14 69 ± 1 03	17 80 ± 0 45	14 50 ± 0 65	15 13
	L	15 21 ± 1 13	17 91 ± 0 44	16 26 ± 0 62	16 65
Schiøtz tension	R	14 44 ± 1 02	17 26 ± 0 49	14 50 ± 0 67	15 51
	L	14 05 ± 1 03	17 10 ± 0 48	15 62 ± 0 63	15 82
Rigidity	R	0223 ± 0014	0281 ± 0021	0226 ± 00094	0246
	L	0227 ± 0012	0294 ± 0021	0239 ± 00098	0258

All values are given as means ± s.e.m dimensions in mm and tensions in mmHg Glaucoma simplex no of eyes R = 16 L = 14 Ocular hypertension no of eyes R = 25 L = 23 Fibrillopathia epitheliocapsularis no of eyes R = 26 L = 23 Average age glaucoma simplex 66 years (49–76) ocular hypertension 63 years (51–76) and fibrillopathia epitheliocapsularis 72 years (59–86)

eyes 0 524 ± 0 002 (Kruse Hansen 1971) In patients with ocular hypertension the corneal thickness is significantly higher than in a normal group (right eyes $P < 0\ 01$ left eyes $P < 0\ 001$) and in the group with glaucoma simplex (right eyes $P < 0\ 01$ left eyes $P < 0\ 001$) In the group with fibrillopathy the corneal thickness is higher than normal but not statistically significant (right eyes $P < 0\ 02$ left eyes $P < 0\ 1$)

In the groups (2) and (3) the standard error of the mean is higher than in both the glaucoma *simplex* group and the normal series probably due to heterogeneity within the two former groups

As regards the chamber depth lens thickness length of vitreous and length of eyeball no significant differences were found between the three groups The average values for the depth of the anterior chamber agree with those given by Törnquist (1953) and Alsbirk (1974) although lower than those of Jansson (1963) The thickness of the lens considering the age of patients is also within the range given in the literature (see e.g Jansson 1963) The values of length of vitreous and length of eyeball are lower than those of Jansson (1963) and Stenstrom (1946) and for some of the groups statistically significant deviations from the data in the literature are found

Ocular tensions and rigidity Table I shows the calculated average values for applanation tension Schiotz tension and rigidity The applanation tensions tend to be higher than the Schiotz tensions although none of the differences are statistically significant. The tensions of the glaucoma simplex group were normal following treatment. The group of ocular hypertension showed average tensions between 17 and 18 mmHg Some of the differences between this group and the other groups are significant.

The rigidity is also higher in the ocular hypertension group the difference between this and the two other groups was probably significant ($P \sim 0.05$)

Correlations of corneal thickness to other ocular dimensions Possible correlations were studied graphically and by computing the correlation and regression coefficients for the three groups and for the total material for right and for left eyes separately The data are illustrated by the matrix in Table II which applies to the total material right eyes upper figures and left eyes lower figures Similar matrices were made for the three subgroups (1–3) and the data were essentially similar In Table II correlation coefficients above 0 24 may be regarded as significant ($P < 0.05$) It may be noted that no correlations could be demonstrated between corneal thickness and the other dimensions

Correlations between depth of anterior chamber lens thickness vitreous and axial length Significant positive correlations were found between depth of anterior chamber and axial length and between vitreous and axial length. A negative correlation was found between depth of anterior chamber and lens thickness (Table II) Other tested correlations were insignificant.

Correlations of dimensions to tension and rigidity There is a positive correlation between corneal thickness and applanation tension ($P \sim 0.05$) The same tendency may be noted for the Schiotz tension, although it is not statistically significant The following equations have been obtained from the total series

$$\text{Appl (mmHg)} = 2\ 43 + 24\ 99\ \text{CCT (mm)}$$

Table II
Correlation matrix

	AC depth	Lens thickness	Vitreous length	Axial length	Age	Appl tension	Schiøtz tension	Schiøtz scale	Rigidity
Corneal thickness	–0 178 0 007	0 032 –0 163	0 129 0 228	0 117 0 172	0 067 –0 030	0 228 0 251	0 174 0 150	–0 180 –0 187	0 137 0 251
AC depth		–0 505 –0 640	0 173 0 273	0 409 0 380	0 111 –0 120	0 199 0 074	0 191 –0 018	–0 189 0 043	–0 044 –0 023
Lens thickness			–0 909 –0 281	0 061 –0 057	0 319 0 429	–0 039 –0 101	–0 038 –0 079	0 019 0 040	–0 060 –0 132
Vitreous length				0 867 0 904	0 244 0 113	–0 081 0 045	–0 157 –0 046	0 148 0 042	–0 379 –0 245
Axial length					0 321 0 247	–0 012 0 033	–0 062 –0 091	0 044 0 033	–0 345 –0 296
Age						–0 151 –0 096	–0 124 –0 096	0 101 0 050	–0 303 –0 294
Appl tension							0 700 0 865	–0 703 –0 947	0 299 0 457
Schiøtz tension								–0 993 –0 979	0 514 0 475
Schiøtz scale									–0 499 –0 440

Correlation coefficients above 0 31 are significant at the 1% level coefficient above 0 24 at the 5% level
Upper figures 67 right eyes lower figures 60 left eyes

(right eyes N = 67) Computed t value for regression coefficient 1 89 ($P < 0\ 05$)

Schiotz (mmHg) = 5 55 + 18 59 CCT (mm)

(right eyes N = 67) Computed t value for regression coefficient 1 42 ($P < 0\ 15$)

There were no correlations between depth of anterior chamber or lens thickness and tensions or rigidity (Table II) A negative correlation exists between vitreous length and axial length on the one hand and rigidity on the other

Correlations to age Lens thickness and axial length are positively correlated to age whereas there is a negative correlation between age and rigidity (Table II) *It must be emphasized that the material comprises mainly old* patients and is therefore rather unsuitable for a study of age correlations

Multiple correlations The measured applanation tension is correlated to the corneal thickness Although the present material is heterogeneous and not composed of normal eyes it was decided to utilize it in an attempt to determine whether other parameters are of importance for the measurement of tension This has been done by computing several multiple correlations of which only a few will be discussed

Appl (mmHg) = 2 67 + 24 93 CCT (mm) − 0 012 Axial length (mm)

The t value for the regression coefficient with respect to corneal thickness is 1 86 ($P < 0\ 05$) the regression with respect to axial length is not significant ($t = -0\ 02$) *The present material suggests therefore that the axial length (size* of eyeball) is of no importance in applanation tonometry

A similar computation for the Schiotz tension gives

Schiotz (mmHg) = 12 23 + 19 63 CCT (mm) − 0 32 Axial length (mm)

t Values for the regression coefficients are 1 49 and − 0 68 both statistically insignificant Thus from the material the axial length would also seem to be of little importance for indentation tonometry

Discussion

The present study was performed after it had been found that the central corneal thickness in a small series of patients with monosymptomatic ocular hypertension was significantly higher than normal (Kruse Hansen & Ehlers

1971) This observation has been confirmed by the present study where a statistically significant higher thickness was found in the group with ocular hypertension than in either the glaucoma simplex group or our normal series (Kruse Hansen 1971) No difference between the glaucoma simplex group and the normal series was found in accordance with the statement of Tomlinson & Leighton (1972) The thickness in the fibrillopathy group is slightly but not significantly higher than normal This might be due to the fibrillopathy and then be correlated with the fact that the mean intraocular pressure in eyes with fibrillopathy is higher than in eyes without fibrillopathy (Aasved 1971)

No significant differences were found between the three subgroups for the other dimensions A general comment on the other parameters is outside the scope of this paper The flatter anterior chamber in simple glaucoma is in accordance with Tornquist & Brodén (1958) and Tomlinson & Leighton (1972)

The average values for applanation and indentation tension are normal again excepting the group with ocular hypertension in which it is increased The ocular rigidity is also higher in this group than in the other groups

The statistical analysis confirmed the previously found (Kruse Hansen 1971) correlation between corneal thickness and applanation tension There was a tendency to a correlation between corneal thickness and Schiøtz tension although not statistically significant

It appears from the calculated correlation coefficients that the central corneal thickness is a parameter which is mainly independent of other ocular dimensions This independence of corneal thickness and the correlation between thickness and pressure would seem to indicate that the thickness is a factor of clinical importance in the evaluation of intraocular pressure This conclusion is in agreement with the findings of reduced thickness in low tension glaucoma and increased thickness in monosymptomatic ocular hypertension From the recently reported experimental study (Ehlers et al 1975) it seems that the influence of the thickness on the applanation tonometer reading is an additional factor of clinical importance

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PROGNOSIS OF PRIMARY RHEGMATOGENOUS RETINAL DETACHMENTS

1 Associations between clinical detachment characteristics subretinal fluid butyrylcholinesterase and visual outcome following scleral buckling procedures

BY

PAUL L. KAUFMAN

Thirty five patients with primary rhegmatogenous retinal detachments were followed for at least 6 months after scleral buckling procedures with subretinal fluid (SRF) drainage, in order to define factors influencing anatomic and visual outcome.
Thirty two cases were surgically reattached three were not. Among the reattached cases final visual acuity was poorer in patients with older age longer standing more extensive detachments detachment of the macula (with or without the development of a visible macular lesion) macular lesions and higher SRF butyrylcholinesterase activity. These factors were themselves interrelated. Follow up duration was only weakly related to final acuity probably because of the long post surgical follow up. Phakic/aphakic status bore little relationship to final acuity. The type or timing relative to drainage of inflammation producing treatment was not related to final acuity.

Key words: butyrylcholinesterase – retina – retinal datachment – subretinal fluid.

In the treatment of primary rhegmatogenous retinal detachment (PRRD) the closure or walling off of all retinal breaks and the production of adequate chorioretinal adhesions are of unquestioned importance. However disease

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related and patient related factors must strongly influence the outcome Identification of such factors is important from both the prognostic and pathophysiologic points of view

The presence of large plasma proteins in subretinal fluid (SRF) reflects abnormal permeability in choriocapillaris Bruch s membrane and retinal pigment epithelium (RPE) which may in turn reflect the pre and post operative health of the choroidal circulation and the RPE and may therefore be prognostically significant (Heath Beck & Foulds 1962 Weber & Wilson 1963 Kaufman & Podos 1973a) The enzyme butyrylcholinesterase (BuChE E C 3 1 1 8 pseudocholinesterase non specific plasma or serum cholinesterase) is a naturally occurring marker for the plasma component in SRF (Kaufman & Podos 1973a b) It is a large molecule (MW $\sim$ 300 000 Juul 1968) and while high levels are found in plasma (Juul 1968) it is absent or present in only minute quantities in human ocular tissues and erythrocytes (Leopold 1961 Leopold & Furman 1971 Kaufman & Podos 1973b) Significant amounts of the enzyme are present in SRF from nearly 90 % of PRRD s and the enzyme level increases with increasing duration and extent of detachment (Kaufman & Podos 1973b)

In the current study relationships are identified between clinical detachment characteristics SRF BuChE activity and visual outcome in 35 PRRD s

Materials and Methods

Thirty five patients with PRRD were studied All underwent scleral buckling procedures with drainage of SRF The criteria for inclusion in the study and for the determination of the clinical detachment characteristics basis demographic data the therapy employed the methods for obtaining SRF and plasma and assaying their BuChE activity and some of the findings correlating BuChE activity with the clinical parameters have been previously reported for these same patients (Kaufman & Podos 1973b)

All thirty five patients were followed clinically for 6 months or longer after their detachment surgery Each patient was examined during follow up office visits by one of four experienced retinal surgeons usually the one who had performed the surgery Fundus examination was by direct/indirect ophthalmoscopy and if the examiner thought is was indicated by biomicroscopy fundus photography or fluorescein angiography From the office records of each visit were determined visual acuity retinal attachment/detachment phakic/aphakic status and the presence or absence of a visible macular lesion

Table I

Final visual acuity vs patient age follow up duration detachment duration and extent and SRF BuChE activity in surgically reattached cases

Group	Final visual acuity	No cases	Patient age (yrs)*	Follow up duration (months)*	Detachment duration (days)**	Detachment extent (clock hours)*	SRF BuChE (% of plasma activity)*
			A All cases				
a	≥ 20/40	10	47 9±5 5	13 5±1 3	14 8	6 6± 7	3 92± 94
b	20/50–20/100	12	64 0±2 7	12 6±1 1	8 1	5 8± 6	2 11± 33
c	≤20/200	9	69 4±7 4	11 1±1 3	34 2	10 7± 3	8 23±1 90
d	≥20/100	22	56 7±3 3	13 0±0 9	10 8	6 1± 5	2 67± 44
			Pa-b< 02	NS	Pb-c< 005	Pa-c< 001	Pa-c< 05
			Pa-c< 05		Pc-d< 01	Pb-c< 001	Pb-c< 01
						Pc-d< 001	Pc-d< 01
			B Cases with macular detachment but without macular pathology				
a	≥20/40	8	51 7±9 2	14 0±2 0	10 9	6 9±1 4	1 08± 32
b	20/50–20/100	7	63 7±4 4	11 3±1 2	10 4	7 0± 5	2 91± 90
c	≤20/200	5	75 4±3 8	10 8±2 1	45 7	10 6± 5	10 10±3 06
d	≥20/100	10	60 1±4 3	12 5±0 9	10 5	6 9± 5	2 36± 37
			Pc-d< 0?	NS	Pb-c< 05	Pa-c< 05	Pa-b< 005
					Pc-d< 05	Pb-c< 001	Pa-c< 05
						Pc-d< 001	Pb-c< 05
							Pc-d< 05

		C Cases with macular detachment and macular pathology					
		5	64 4±3 9	14 4±2 0	5 8	4 0± 4	0 98± 32
	20/50–20/100						
	≤20/200	4	62 0±16 4	11 5±1 9	25 0	10 9± 4	5 89±1 63
			NS	NS	$P<05$	$P<001$	$P<025$
		D Cases without macular detachment or macular pathology					
	≥20/40	7	46 3±7 1	13 3±1 8	16 9	6 6±1 0	4 14±1 01
		E All aphakic cases					
a	≥20/40	5	59 2±3 2	13 4±1 3	13 2	8 0± 9	5 13±1 14
b	20/50–20/100	7	63 4±2 5	13 4±1 3	7 3	5 1± 7	1 48± 44
c	≤20/200	2	74 0±0 0	10 0±4 0	29 0	11 0±1 0	6 84±2 80
d	≥20/100	12	61 7±2 0	13 4± 9	9 4	6 3± 7	3 21± 73
			Pa–c< 01	NS	Pb–c< 001	Pa-b< 05	Pa–b< 02
			Pb–c< 005		Pc–d< 001	Pb–c< 005	
			Pc–d< 001			Pa–c< 10	
						Pc–d< 005	
		F Aphakic cases without macular detachment or macular pathology					
	≥20/40	4	57 8±3 7	13 8±1 7	17 8	7 6±1 1	6 09± 79
		G Aphakic cases with macular detachment but without macular pathology					
	≥ 20/40	1	65	12	4	9 5	1 27
	20/50–20/100	3	60 3±2 9	13 7±1 7	9 0	6 5±1 0	3 19± 41
	≤20/200	1	74	6	28	10	4 04

Table I (continued)

Group	Final visual acuity	No cases	Patient age (yrs)*	Follow up duration (months)*	Detachment duration (days)**	Detachment extent (clock hours)*	SRF BuChE (% of plasma activity)*
		H Aphakic cases with macular detachment and macular pathology					
	20/50–20/100	4	65 8±3 8	13 3±2 1	6 3	4 0± 5	0 83± 36
	≤20/200	1	74	14	30	12	9 64
		I All phakic cases					
a	≥20/40	5	36 6±7 8	13 6±2 5	16 4	5 2± 8	1 32± 22
b	20/50–20/100	5	64 8±6 1	11 4±2 1	9 4	6 7± 8	2 48± 26
c	≤20/200	7	68 1±9 6+	11 4±1 5	36 9	10 6± 4	8 63±2 38
			*P*a-b< 02	NS	*P*b-c< 05	*P*a-c< 001	*P*a-b< 01
			*P*a-c< 05			*P*b-c< 005	*P*a-c< 02
							*P*b-c< 05
		J Phakic cases without macular detachment or macular pathology					
	≥20/40	3	31 0±11 1	12 7±4 1	15 6	5 2±1 5	1 54± 13

* Arithmetic means ± SEM
** Geometric mean
+ One patient age 14 in this group Mean age of other patients accordingly greater
P values determined by the two tailed Student t test refer to the difference between the means for the subscripted groups
For detachment duration the t test was applied to the mean logarithms the antilogs of which are the listed geometric means
NS = not significant

The statistical methods used to evaluate the data are indicated with the presentation of the results The results involving SRF BuChE were the same whether enzyme activity was expressed as absolute levels (International Units/ml Kaufman & Podos 1973a) or as a percentage of the plasma enzyme activity Since the enzyme activity in SRF is a quantitative indicator of the plasma component (Kaufman & Podos 1973a b) only the latter is presented

Results

Retinal reattachment

In 31 eyes (89 %) the retina has remained attached after the initial procedure One eye required a second operation 3 months after the first for relief of localized pre retinal retraction with SRF accumulation both far removed from the macula The retina has remained attached for 6 months following the second operation Thus in 32 eyes (91 %) the retina was surgically reattached Three eyes have remained detached after the initial surgery All developed massive vitreous retraction (MVR) and were not considered candidates for further surgery The 32 cases which were surgically reattached did not differ strikingly with regard to length of follow up patient age prevalence of aphakia type (cryotherapy or diathermy) or time of application (pre or post SRF drainage) of inflammation producing treatment duration or extent of detachment or SRF BuChE activity from the three cases which were not reattached If reattachment after one procedure is taken as the criterion for surgical success the 31 successful cases did not differ from the four unsuccessful ones with regard to these parameters

Visual acuity

The distribution of the cases according to detachment duration was positively skewed with a long tail toward the longer durations Therefore a logarithmic transformation (Mainland 1970) was employed in analyses involving detachment duration

Final visual acuity was taken as that attained at the last follow up visit In no case did acuity decline once it had reached its maximum In the analysis of the final acuities the three cases which were not reattached were excluded Also excluded was one surgically reattached case with a long standing strabismic amblyopia which predated the detachment

The 31 surgically reattached cases remaining were divided according to

final acuity into those seeing 20/40 or better those seeing 20/50 to 20/100 and those seeing 20/200 or worse Poorer final acuity was associated with older patient age greater detachment duration and extent and higher SRF BuChE activity (Table I A) The three acuity groups did not differ significantly in the prevalence of aphakia or the type (cryotherapy or diathermy) or timing (pre or post drainage) of inflammation producing treatment (χ test) There seemed to be a slight tendency for the better seeing patients to have been followed longer but this was not significant

Nine eyes had visible macular lesions consisting of fibroplasia (4) pigment fallout from cryotherapy (2) cyst (2 in one of these a subretinal hemorrhage preceded the development of the cyst) and demarcation line (1) In one eye with macular fibroplasia and in one with a macular cyst the lesion was present

Table II

Final visual acuity vs macular pathology and macular detachment in surgically reattached cases

Final visual acuity	No cases	Frequency of cases with		
		Macular detachment	Macular pathology	Macular detachment among cases without macular pathology
		(A All cases (n = 31)		
≥20 40	10	3/10	0/10	3/10
≤20 50	21	21/21	9/21	12/12
		P< 0005	P< 05	P< 005
		B Phakic cases (n = 17)		
≥20 40	5	2/5	0/5	2/5
≤20/50	12	12/12	4/12	8/8
		P< 025	NS	P< 10
		C Aphakic cases (n = 14)		
≥20/40	5	1/5	0/5	1/5
≤20/50	9	9/9	5/9	4/4
		P< 025	NS	P< 10

P values determined by the chi squared method refer to the difference between the acuity groups in the frequency of occurrence of the clinical parameter
NS = not significant

Table III

Patient age vs macular detachment in surgically reattached cases

Macular detachment/ attachment	No cases	Patient age (years – mean ± SEM)	
	A All cases (n = 31)		
Macula detached	24	64.5±3.3	$P < 05$
Macula attached	7	46.3±, 1	
	B Cases without macular pathology (n = 22)		
Macula detached	15	65 ?±3 6	$P < 05$
Macula attached	7	46.3±7 1	

P values determined by the two tailed Student t test refer to the difference between the mean a es of patients with and without macular detachment.

at the time of surgery but presumably not prior to the detachment in the other seven eyes the lesions developed during the follow up period For purposes of analysis these nine eyes were grouped together as having macular pathology As expected macular pathology was significantly more prevalent among eyes with 20/50 or worse final acuity than among those seeing 20/40 or better indeed none of the cases in the latter group demonstrated such pathology (Table II A)

In 24 of the 31 eyes the macula had been detached at the time of surgery Macular detachment was more prevalent among the 20/50 or worse groups (it had occurred in all of these eyes) than among the 20/40 or better cases even in cases without macular pathology (Table II A) All nine cases of macular pathology occurred among the 24 cases in which the macula had been detached (Table II A) Although the data do not prove that the prevalence of macular pathology was significantly greater when the macula had been detached ($\chi^2 = 2.1027$ $P < .20$) they sugge t that macular detachment predisposed to the development of macular pathology Patients in whom the macula had been detached were significantly older than those in whom it had not (Table III A B) There was no difference in age between patients with and without macular pathology whether one considers all 31 cases or only the 24 with macular detachment. There were no significant differences in the prevalence of aphakia the type or timing of inflammation producing treatment, duration or extent of detachment, SRF BuChE activity or length of follow up

between cases with and without macular pathology or between cases with and without macular detachment

Among cases with macular detachment both with and without a macular lesion poorer final acuity was associated with older patient age greater detachment duration and extent high SRF BuChE activity and a slight but non significant tendency toward shorter follow up (Table I B C) No differences in the prevalence of aphakia or the type or timing of inflammation producing treatment existed between acuity groups

To determine whether the poorer final acuities in the older patients were related to cataractous lens changes the data for phakic and aphakic patients were analyzed separately The proportion of cases seeing 20/200 or worse was *somewhat but not significantly higher among the phakic cases (7/17) than* among the aphakes (2/14 $P < 20$) The proportion of cases seeing 20/40 or better was essentially the same in both groups (5/17 vs 5/14) The relationships between final acuity and patient age follow up duration detachment duration and extent SRF BuChE activity macular detachment and macular pathology were generally similar in both groups (Table I E J) Thus lens changes could not account for the results

In Table IV each acuity group has been subdivided according to the macular factors Within each acuity group the ages of the subgroups were comparable but the subgroup with the more insulted maculas (detachment vs no detachment pathology vs no pathology) tended to have shorter standing detachments and lower SRF BuChE activity In the 20/50 to 20/100 group the detachments with the more insulted maculas were also of lesser extent Furthermore the 20/50–20/100 patients especially those with both macular detachment and pathology had shorter duration and smaller detachments and lower enzyme levels than ≥ 20/40 patients especially those with neither macular detachment nor pathology These observations were especially noticeable among the aphakes (Table I E F and H) These findings could reflect that patients whose maculas were not detached and in whom central vision was never lost *were less motivated to seek prompt medical attention than the* 20/50–20/100 group in which central vision was lost relatively early By seeking prompt care and having their maculas quickly reattached this latter group may have had a better chance of saving some macular function than the ≤ 20/200 group whose maculas were also detached but who waited longer Another reasonable inference is that if the macula did not detach an excellent visual outcome was assured regardless of other parameters The findings might also indicate that the parameters compensated for one another to some degree in influencing final acuity The age differences between the acuity groups complicates these assessments

Table IV

Relative roles of macular detachment/pathology patient age detachment duration and extent and SRF BuChE activity in accounting for final visual acuity in surgically reattached cases

Group	Final visual acuity	Macular detachment Macular pathology	No cases	Patient age *(yrs)*	Detachment duration *(days)**	Detachment extent *(clock hours)**	SRF BuChE (% of plasma* activity)
a	≥20/40	−/−	7	46 3±7 1	16 8	6 6±1 0	4 14±1 01
b	≥20/40	+/−	3	51 7±9 2	10 8	6 7±1 4	1 08± 32
c	20/50–20/100	+/−	7	63 7±4 4	10 4	7 0± 5	2 91± 20
d	20/50–20/100	+/+	5	64 4±3 2	5 8	4 0± 4	0 98± 32
e	≤20/200	+/−	5	75 4±3 8	45 7	10 6± 5	10 10±3 06
f	≤20/200	+/+	4	62 0±16 4+	25 0	10 9± 4	5 89±1 63
				Pa–b	NS	NS	< 025
				Pa–d	< 06	< 05	< 02
				Pc–d	< 05	< 001	< 001

* Arithetic means ± SEM

** Geometric means

One patient age 14 in this group Mean age of other patients accordingly higher

P values determined by the two tailed Student t test refer to the difference between the means for the subscripted groups

For detachment duration the t test was applied to the mean logarithms the antilogs of which are the listed geometric means

NS = not significant

Discussion

None of the parameters investigated seemed related to whether or not the retina was reattached by the surgery However very few of the cases were not reattached so one can only say that no striking differences were noted

In the surgically reattached cases poorer final acuity was associated with greater patient age greater detachment duration and extent greater SRF BuChE activity and with the presence of preoperative macular detachment and postoperative macular pathology If the macula had not been obviously insulted (no detachment no pathology) an excellent visual acuity was assured If the macula had been insulted in a clinically apparent manner (detachment with or even without visible pathology) the degree to which final vision was compromised depended not only on the clinically obvious macular insult but also upon the other parameters These parameters therefore presumably *related in some way to anatomic and/or functional macular changes which* were not clinically detectable by the examination techniques employed

Although it may require many months for central acuity to reach a maximum following retinal reattachment (Schepens & Regan 1965 Duke Elder & Dobree 1967) only a weak non significant relationship between final acuity and follow up duration was noted in the present cases The length of follow up (mean = 12 5 mos) and the requirement that each case be followed for at least 6 months after surgery probably insured that by and large sufficient time was allowed for the best possible acuity to be attained

Lens opacities could not explain the findings since the relationships between final acuity and the various parameters were similar in phakic and aphakic patients

In two of the nine cases with macular abnormalities the lesion was thought to be a consequence of cryotherapy Furthermore the application of inflamma tion – producing treatment before SRF drainage was associated with increased SRF BuChE activity (diathermy more so than cryotherapy) although it is not known whether this was causal or due to a biased selection of cases for treatment *prior to drainage (Kaufman & Podos 1975b)* Therefore a relation ship between final acuity and the type of inflammation producing treatment or the time of its application relative to SRF drainage was looked for None was found

The findings presented illustrate the difficulties in assessing what determines visual outcome in surgically reattached PRRD The parameters related to final acuity are themselves interrelated so one does not know which really matter or the pathophysiology involved However by applying multiple linear regression and analysis of variance to the raw data one can isolate the

influence of each parameter and also see whether final acuity can be explained – or predicted – by all the parameters acting in concert This will be the subject of a future communication

Acknowledgments

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*Ophthalmic Literature

This journal has hitherto been published by the British Medical Association but for financial reasons they are discontinuing publication Starting from Vol 29 September 1975 publication will be undertaken by the Institute of Ophthalmology University of London believing as we do that it is of great value for ophthalmologists and for the furtherance of research We hope the journal will continue to prosper in the future and any profits that may be made will be devoted to ophthalmic research

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The European Ophthalmic Pathology Society held its 14th Annual Meeting in Oslo Norway June 3–6 1975 Dr J A C Wadsworth Durham North Carolina was the honoured guest A further eight European guests and 32 members attended the meeting which was perfectly organized by Professor and Mrs K Arnesen The scientific sessions were held at the University of Oslo Institute of Preclinical Odontology Dr S Ry Andersen Denmark was elected President and Dr O A Jensen Denmark Corresponding Secretary Drs A S Henriquez (Spain) and D Toussaint (Belgium) were elected new members The next m eting will be a joint meeting with the Verhoeff Society in Washington Dr L E Zimmerman being the Organizing Secretary

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IN VITRO STUDIES ON PEROXIDASE MOVEMENT IN THE EPITHELIUM OF PROSTAGLANDIN TREATED RABBIT CILIARY BODIES

BY

OLAV ØYVIND PEDERSEN and
ASBJØRN M TØNJUM

The movement of horseradish peroxidase (HRP) in the epithelium of isolated rabbit iris/ciliary body preparations has been studied with the electron microscope HRP was applied at the stromal side of the epithelium and was left for 60 and 120 min The distribution pattern of HRP found in the epithelium of the iridial and ciliary processes is consistent with *in vivo* studies i e the progression of HRP is blocked at the site of the *zonula occludens* of the superficial epithelium The HRP distribution pattern found in the iris epithelium indicates that also the superficial epithelial cells of this epithelium are girdled by *zonulae occludentes* Specimens treated with prostaglandins E_1 E_2 and $F_{2\alpha}$ showed no change in the epithelial distribution pattern of HRP and the occluding zonules were found to be intact

Key words ciliary epithelium – permeability – peroxidase – prostaglandins – *in vitro* – microscopy electron – rabbit

The superficial (non pigmented) epithelial cells of the ciliary processes are girdled by a system of tight junctions (*zonulae occludentes)* comprising an important structure of the blood aqueous barrier When horseradish peroxidase

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(HRP) is introduced into the bloodstream as a tracer it permeates the vessels and the stroma of the ciliary processes and the movement towards the posterior chamber is finally blocked by the tight junctions of the *superficial epithelium* (Shiose 1970 71 Smith 1971 Vegge 1971) During acute inflammation of the anterior uvea produced experimentally by intravitreal injection of antigen in the rabbit this epithelial diffusion barrier to peroxidase is broken (Pedersen 1973 74) However in experimental uveitis the situation is very complex and many inflammatory factors are probably involved To evaluate the direct effects of such factors on the epithelial protein diffusion barrier in the ciliary processes *in vitro* studies have to be performed

The purpose of the present paper is to describe an *in vitro* technique whereby the movement of tracer substances (like HRP) in the ciliary epithelium can be studied with the electron microscope The prostaglandins E_1 E_2 and F_2 are regarded as being involved in the pathogenesis of uveitis and possible effects of these prostaglandins on the movement of HRP in the ciliary and iridial epithelium have been studied

Material and Methods

Eyes from albino rabbits weighing 3–3 5 kg were used The animals were killed with an overdose of sodium pentobarbital The eyes were immediately enucleated and the iris and ciliary body were removed in one piece under a dissecting microscope while submersed in Krebs Ringer bicarbonate solution (pH 7 4) with glucose (5 mg/ml) This nutrient solution was used throughout the experiments

Experiments

The iris and ciliary body specimen was cut into sectors and clamped between two lucite chambers both having a volume of about 3 ml The side of each *chamber facing the other had a circular aperture of 3 mm in diameter* This aperture was completely covered by the ciliary body/iris specimen The stromal side of the ciliary body and the anterior surface of the iris faced one chamber while the *epithelial side with the ciliary and iridial processes* and the posterior surface of iris faced the other The chambers were filled with the nutrient solution either alone or with the addition of the tracer and/or test substances The solutions were stirred by teflon coated magnets rotating at a rate of 375 rev/min The set up was the one employed by Tønjum (1974)

The following experiments were done

1 Horseradish peroxidase (HRP) (Sigma type II) was added to the stromal side chamber at a concentration of 2 mg/ml This was the standard concentration of HRP in this study The tissues were exposed to this solution for 1 or 2 hours The specimens were then removed and processed as described below

2a Nutrient solution with prostaglandin (PG) E_1 or E_2 was placed in both chambers for 30 min The fluids were then replaced with fresh and identical solutions which were left for another 30 min Then the solution on the epithelial side was renewed i e after 60 min At the same time the solution on the stromal side was also renewed but this time with the addition of HRP After another 30 min these solutions were renewed and left for 30 min

Thus these experiments lasted for 120 min and the HRP exposure time was 60 min

In some experiments $PGF_{2\alpha}$ was used These solutions were now renewed only *once i e after 60 min Otherwise these experiments were run identically to* those with PGE_1 and E_2

In one experiment with PGE_2 autologous serum was used as the basic solution on the stromal side The blood was obtained by heart puncture during general anaesthesia with sodium pentobarbital immediately before enucleation of the eyes This experiment was run at 35 C but was otherwise similar to the experiments with PGE_1 and E_2 described above

2b For control of the possible PG and ethanol effects (see below) some experiments were run at room temperature using the nutrient solution as before on both sides with and without 1 % ethanol but with the exclusion of PGs

Prostaglandin solutions

The PG solutions were made up in the following way PGE_1 and E_2 were dissolved in absolute ethanol at a concentration of 10 mg/ml (stock solutions) One ml of this solution was quickly added to 9 ml of an aqueous solution of sodium carbonate (0 2 mg/ml) and was further diluted 10 times with the nutrient solution This solution was used in the experiments and contained 0 1 mg/ml PGE_1 or E_2 per ml and 1 % ethanol The $PGF_{2\alpha}$ was dissolved directly in the nutrient solution at a concentration of 1 mg free acid per ml

The stock solutions were stored at − 20 C and fresh solutions were made up immediately before the experiments were done

Processing of the tissues

The tissues were prefixed in 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 for about 2 hours and washed over night in 0 1 M phosphate buffer

pH 7 4 containing 5 % sucrose Incubation was done in tris HCl buffered diaminobenzidine H O solution pH 7 6 (Karnovsky 1967) for about 30 min The tissues were postfixed in 1 % OsO_4 in Millonig s phosphate buffer dehydrated in ethanol treated with propylene oxide and embedded in Epon 812

Ultrathin sections were made with an LKB Ultrotome They were examined uncontrasted or contrasted with an aqueous solution of uranyl acetate and/or alkaline lead Electron micrographs were taken with a Siemens Elmiskop 1A

Results

Regarding the nomenclature of the epithelial cell layers of the ciliary and iridial processes and the posterior surface of iris the terms basal and superficial are used as pertaining to the stroma and to the posterior chamber respectively Thus the epithelial cells facing the posterior chamber are called the superficial epithelial cells and those lining the stroma are called the basal epithelial cells The abbreviation "HRP is used interchangeably for horseradish peroxidase and its reaction product

1 HRP movement in control specimens

No significant differences were found either in the morphology of the epithelia or in the HRP distribution whether the specimens were clamped for 1 versus 2 hours After 1 hour HRP had permeated the stroma of the ciliary body and the iris and was found in the epithelia

In the iridial and ciliary processes HRP was found in the stroma in the lateral intercellular spaces between the basal epithelial cells and in the intercellular space between the basal and the superficial epithelium HRP progression was blocked basally in the lateral intercellular spaces of the superficial epithelium i e at the location of the tight junctions (*onulae occludentes)* of this epithelium The lateral intercellular spaces beyond the junction were free of tracer (Fig 1)

Some HRP containing vesicles were found both in the superficial and the basal epithelial cells but the content of these vesicles was not released *to the* internal limiting membrane or to the lateral intercellular spaces of the superficial epithelium in sufficient amount to be detected

In the iris epithelium the distribution pattern of HRP was the same as in the processes except that occasionally a longer section of the basal part of the lateral intercellular space of the superficial epithelium was filled HRP progression along this intercellular space had come to an abrupt stop at a waist which may well correspond to an impermeable *zonula occludens*

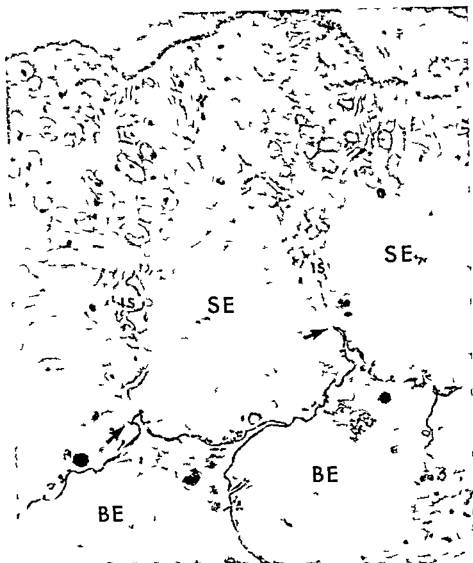


Fig 1

HRP distribution in the epithelium of a ciliary process from a specimen that had not been treated with PG HRP is seen in the lateral intercellular spaces of the basal epithelium (BE) and in the intercellular space between the basal and the superficial epithelium (SE) The progression of HRP has been blocked at the basis of the inter cellular spaces of the superficial epithelium (arrows) The lateral intercellular spaces (is) of the superficial epithelium is free of tracer Lead. x 9 000

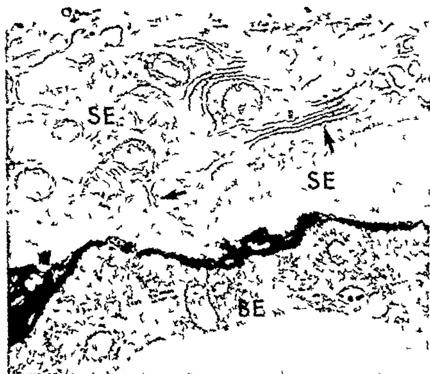


Fig 2

PGF Iridial process HRP is seen in the intercellular space between the basal (BE) and the superficial (SE) epithelium The lateral intercellular space (arrows) between the superficial epithelial cells is free of tracer Lead ×28 500

2. HRP movement in prostaglandin treated specimens

In the tissues that were subjected to PGE$_1$ E and F the same distribution of HRP as in the control specimens was found in the epithelium of the iridial (Fig 2) and the ciliary processes (Fig 3) as well as in the iris epithelium (Fig 4) There was no change in the width of the intercellular spaces as compared with the untreated tissues The intercellular spaces between the basal epithelial cells as well as the space between the basal and the superficial epithelium did not show dilations However minor dilations of the lateral intercellular spaces of the superficial epithelium were occasionally found both in the untreated and in the PG treated tissues This may be due to hypertonicity of the glutaraldehyde fixative (450 mOsmol/l)

In favourable sections of the tight junctions (*zonulae occludentes*) of the superficial epithelium of the ciliary and iridial processes these were found to be

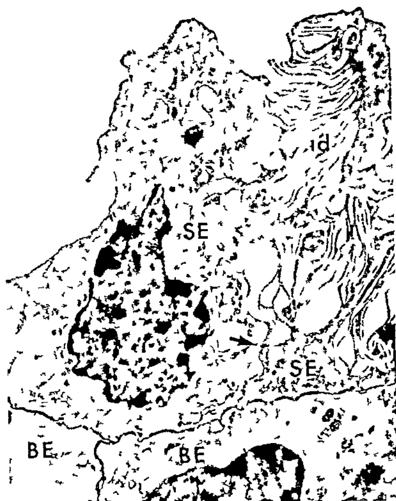


Fig 3

PGE Ciliary process The distribution of HRP is the same as in Figs 1 and 2 HRP is not found in the lateral intercellular space (arrow) of the superficial epithelium or in the interdigitations (id) Uranyl and lead × 10,200

typical pentalayers with a centre to centre distance between the inner leaflets of the cell membranes of about 13 nm (Fig 5) Thus the PGs did not seem to affect either the morphology of the tight junctions or their ability to stop the progression of HRP along the intercellular space

In the specimens that had been subjected to 1 % ethanol without PGs no change in the morphology or the HRP distribution of the epithelia was found as compared with the other controls

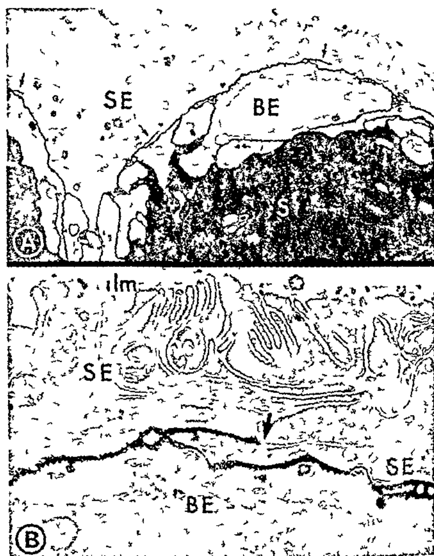


Fig 4

PGE₀ Iris A HRP is found in the stroma (S) between the basal (BE) epithelial cells and in the intercellular space between the basal and the superficial (SE) epithelial cells The basal parts of some of the lateral intercellular spaces of the superficial epithelium have also been filled (arrows) Lead × ₀900 B Detail from upper right in Fig 1A The progression of HRP has been stopped at a narrow waist (arrow) which probably corresponds to an impermeable *onula occludens* The internal limiting membrane (ilm) and the interdigitations are free of tracer Lead × 2₅100

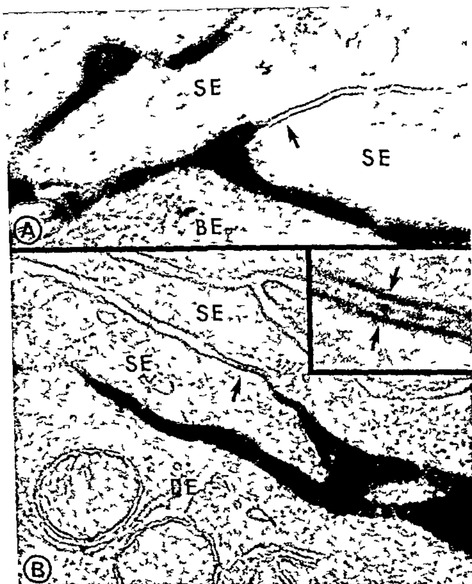


Fig 5

PGE Iridial processes The progression of HRP has been blocked at the basis of the lateral intercellular spaces of the superficial epithelium (SE) by the *onula occludens* (arrows) Basal epithelial cells (BE) A Lead × 120 000 B Lead × 95 100 Inset Detail from *onula occludens* indicated by arrow in Fig 5B It is a typical pentalayer The internal leaflets of the cell membranes are clearly seen (arrows) while the fusion line is less distinct The centre to centre distance between the internal leaflets measures about 13 nm Lead × 430 000

Discussion

The distribution of peroxidase reaction product in the epithelium of the ciliary and iridial processes as demonstrated by the present *in vitro* technique is consistent with findings obtained after intravenous injection of peroxidase in different living animals (Shiose 1970 Smith 1971 Vegge 1971 Pedersen 1973 74 Uusitalo Palkama & Stjernschantz 1973) Therefore the present model is supposedly useful when studying the influence of various agents on the permeability of the ciliary epithelium to proteins In addition the present study indicates that the superficial epithelial cells of the iris like those of the iridial and ciliary processes are girdled by *zonulae occludentes* that are impermeable to HRP

In a previous *in vivo* study a barrier against diffusion of peroxidase from the iridial and ciliary processes through the stroma of the iris and the ciliary body base was demonstrated (Pedersen & Tønjum 1975) The duration of these experiments was 30 and 60 min In the present study peroxidase was found in the processes and the epithelium within 60 min after having diffused through the iris stroma and the ciliary body base Thus the movement of peroxidase is more rapid in the *in vitro* experiments This may be due to some swelling of the intercellular tissue caused by the experimental conditions

After treatment of the tissues with PGE_1 E and F the epithelial distribution pattern of HRP was not altered and the tight junctions were found to be intact

Prostaglandins have been regarded to be involved in the pathogenesis of certain forms of uveitis Thus E and F prostaglandin like activity has been demonstrated in the aqueous humour of patients with untreated acute anterior uveitis (Eakins et al 1972a) Moreover high levels of PGE_1 have been found in the aqueous humor of rabbits with experimental immunogenic uveitis (Eakins et al 1972b) In addition topical and intracameral administration of PCs can induce some of the characteristics associated with ocular inflammation i e constriction of the pupil hyperaemia and breakdown of the blood aqueous barrier (Waitzman & King 1967 Beitch & Eakins 1969 Kass et al 1972)

In experimental uveitis in the rabbit produced by a single intravitreal injection of antigen the epithelial diffusion barrier to proteins is broken In such eyes intercellular leakage of peroxidase through the epithelium as well as damage of the epithelial cells of the iridial and the ciliary processes have been demonstrated (Pedersen 1973 74) However the mechanism by which the blood aqueous barrier is broken both in experimental uveitis and after administration of prostaglandins remains uncertain Neufeld & Sears (1973) claim that the site of action of PGE_2 is at the tight junctions of the superficial (non

pigmented) epithelium of the ciliary body After topical application of PGE_2 to rabbit eyes the protein content of the aqueous humour increased more rapidly in the posterior than in the anterior chamber However morphological studies were not performed Green (1973) found some increase of the hydraulic flow in isolated rabbit ciliary bodies treated with PGE_1 E_2 and F

Our study indicates that the prostaglandins do not affect the epithelial *diffusion barrier* to the small protein horseradish peroxidase. In out set up the hydrostatic pressure was the same on both sides of the epithelium which is not the case *in vivo* However the magnitude of the pressure gradient across the epithelium is disputable and has not been adjusted in the present study Under these conditions we have not been able to demonstrate any breakdown of the tight junctions under the influence of PGs As mentioned above breakdown of these junctions by direct effects of PGs has been suggested to occur (Neufeld & Sears 1973) During the dissection of the preparations PGs might be released However this had no influence on the barrier studied in the present work We are inclined to explain the destruction of the epithelial barrier by prostaglandins *in vivo* as being secondary to vascular changes in the ciliary and/or iridial processes There is some evidence that the vascular permeability in the anterior uvea is increased under the influence of PGs (Whitelocke & Eakins 1973 Cole 1974 Tamura 1974 Pedersen 1975a b)

Acknowledgements

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Prostaglandins are regarded as being involved in ocular inflammations (for references see Neufeld & Sears 1973) To clarify pathophysiological mechanisms involved in uveitis a thorough understanding of the ocular effects of prostaglandins seems to be essential Aqueous flare due to increased protein content of the aqueous humour is an important symptom in uveitis Topical administration of prostaglandins E_1 and E_2 to rabbit eyes can induce some of the characteristics associated with acute anterior uveitis Among other things the prostaglandin reaction involves breakdown of the blood aqueous barrier to proteins (Waitzman & King 1967 Beitch & Eakins 1969 Kass et al 1972)

The purpose of the present work was to study with the electron microscope morphological changes in structures related to the blood aqueous barrier in the ciliary body of rabbit eyes treated topically with prostaglandins E_1 and E_2 In an effort to judge changes in morphology in terms of protein permeability of the barrier horseradish peroxidase has been used as a tracer on the electron microscopic level

Material and Methods

Six healthy albino rabbits weighing about 3 kg were used Before experimentation the eyes were found normal determined with slit lamp and ophthalmoscopic examination

Prostaglandins (PG) E_1 or E_2 were dissolved directly in 0 2 M phosphate buffer pH 7 0 at a concentration of 10 mg/ml

0 5 ml of the solution containing PGE_1 was added dropwise to the conjunctival cul de sac of the left eyes of two rabbits in the course of 5 min The left eyes of two other rabbits received the same amount of the PGE_2 solution

Both eyes of two rabbits served as controls An amount of 0 5 ml (about 10 drops) of the solvent without PG was applied topically to both eyes of one of these rabbits while nothing was applied to the conjunctiva of the other animal

Sixty minutes after the conjunctival application three of the PG treated animals were injected intravenously with 300 mg commercial horseradish peroxidase (Sigma type II) dissolved in 5 ml sterile Ringer solution After another 30 min i e 90 min after the PG application the animals were killed with an overdose of sodium pentobarbital One of the PGE_1 treated animals was injected intravenously with 5 ml sterile Ringer solution without peroxidase 60 min after the application and the animal was killed after another 30 min

The control animal that was treated topically with phosphate buffer was injected intravenously with 520 mg peroxidase 60 min later and was killed

after another 30 min The other control animal received 460 mg peroxidase and was killed 30 min later

The eyes were enucleated immediately after the animals were killed They were opened broadly at the equator and immersed in 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 They were prefixed for about 2 hours During the fixation the eyes were divided into two parts at the equator and the lens was removed from behind The anterior segment was cut meridionally into broad sectors (3–4 mm) The tissue was washed overnight in 0 1 M phosphate buffer pH 7 4 containing 5 % sucrose They were then cut into thin slices (0 5–1 mm) The specimens included iris ciliary body cornea and peripheral parts of the sclera They were incubated in tris HCl buffered diaminobenzidine H O solution pH 7 6 (Karnovsky 1967) for about 30 min The specimens were then postfixed for 1 1/2 hours in 1 % OsO_4 in Millonig s phosphate buffer dehydrated in ethanol treated with propylene oxide and embedded in Epon 812

Some specimens from each of the eyes were not incubated but were otherwise processed in the same way as described above

One μm thick sections for light microscopy were examined either unstained or stained with toluidine blue

Ultrathin sections from iridial and ciliary processes were cut on an LKB Ultrotome Electron micrographs were taken with a Siemens Elmiskop 1A Uncontrasted sections as well as sections contrasted with an aqueous solution of uranyl acetate and/or alkaline lead were examined

Results

In the following the terms iridial and ciliary processes are used in accordance with Kozart (1968) Regarding the nomenclature of the epithelium of these processes the terms basal and superficial are used as pertaining to the stroma and to the posterior chamber respectively The abbreviation HRP is used interchangeably for peroxidase and its reaction product.

1 Control eyes

The movement of HRP from the stroma of the processes through the intercellular spaces of the epithelium were blocked by the *onulae occludentes* at the basis of the superficial epithelial cells The lateral intercellular spaces of the superficial epithelium were free of tracer This is consistent with previous observations in rabbits (Pedersen 1973 74 Uusitalo Palkama & Stjernschantz

1973) in mice (Shiose 1970 71 Smith 1971) and in monkeys (Vegge 1971) No significant dilations of the epithelial intercellular spaces or the ciliary channels were observed either in the iridial or the ciliary processes The HRP distribution and the structure of the epithelium of the ciliary processes were identical to that found in the posterior parts of these processes in the PG treated eyes (Fig 3)

The structure of the fenestrated vessels in the processes was in accordance with previous studies (Holmberg 1959 Pappas & Tennyson 1962 Taniguchi 1962) and will not be described here No haemorrhages or platelet reaction were observed in the processes of the control eyes

2 PG treated eyes

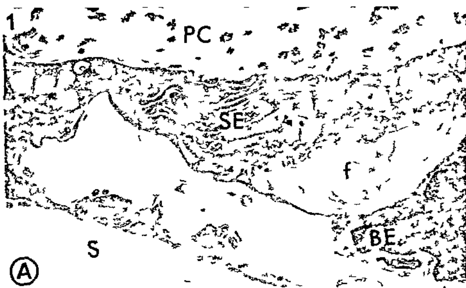
The morphological changes were essentially the same in all PG treated eyes irrespective of whether PGE_1 or E_2 was used At the time of enucleation hyperaemia of the conjunctiva and iris was observed and intense aqueous flare was present in the anterior chamber By dissection of the eyes haemorrhages were observed in iridial processes and in anterior parts of ciliary processes

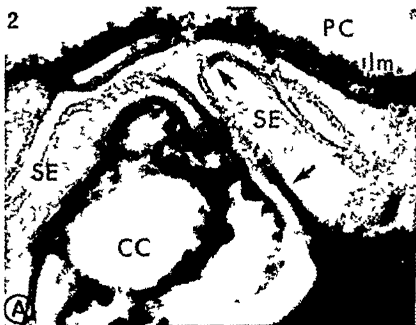
Epithelium Marked structural alterations were found in the epithelium of the iridial processes The basal epithelial cells were partly transformed into thin cytoplasmic strands giving the epithelium a vacuolized appearance (Figs 1A B) The cells were also flattened and frequently large dilations of the lateral intercellular spaces of the basal epithelium were found Dilations of the ciliary channels and partial separation of the two epithelial cell layers were observed (Fig 1) Occasionally the superficial epithelium was missing (Fig 1B) Fibrin like material was demonstrated in the intercellular spaces of the epithelium as well as in the posterior chamber (Fig 1A) In contrast to this only minor dilations of the intercellular spaces were found in the epithelium of the ciliary processes

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Fig 1

Epithelium of iridial processes PGE The epithelium is flattened and the basal epithelial cells (BE) are partly transformed into thin cytoplasmic strands giving the epithelium a vacuolized appearance A In the dilated intercellular space between the basal and the superficial (SE) epithelial cells a fibrin like material (f) is present This is also found in the posterior chamber (PC) Stroma (S) Uranyl and lead × 16 00 B The superficial epithelium has been desquamated The basal epithelium is facing the posterior chamber The posterior chamber and the vacuoles in the epithelium contains a moderately electron dense granular substance Beneath the epithelium closely packed erythrocytes (Er) are present in the stroma Uranyl and lead × 6 900


1
PC
SE
f
S
BE
A
PC
BE
B


2
PC
ilm
SE
SE
CC
A
PC
SE
SE
B

In incubated specimens from animals injected with peroxidase the tracer could in some places be followed continuously from the intercellular space between the two cell layers past the site of the *zonula occludens* throughout the lateral intercellular spaces of the superficial epithelium to the posterior chamber (Figs 2A B) In these regions the tracer also stained the internal limiting membrane These observations indicate that a rupture of the *zonulae occludentes* had taken place or that the junction had become permeable to HRP Leakage of HRP through the intercellular spaces of the epithelium was observed in the iridial processes and in the anterior parts of the ciliary processes In the posterior parts of the ciliary processes the *zonulae occludentes* were found to be intact blocking the movement of the tracer towards the posterior chamber (Fig 3) Occasionally rupture of the *zonula occludens* was observed in the iridial processes The lateral intercellular space of the superficial epithelium at these sites was dilated and contained tracer material

No significant increase in the number of HRP containing vesicles was found in the superficial epithelial cells of the PG treated eyes as compared with the controls Thus it seems clear that the tracer enters the posterior chamber through the intercellular spaces of the epithelium and that vesicular transport is of minor significance in this respect

In incubated specimens from the PGE_1 treated animal that did not receive HRP no extracellular reaction product was demonstrated However the morphological changes were the same as in the other PG treated eyes

Vessels Marked oedema in particular of the iridial processes was observed The presence of haemorrhages was confirmed by microscopy In some areas of the iridial processes the stroma was virtually packed with erythrocytes (Fig 1B) The lumen of the vessels as well as the stroma contained a moderately electron dense granular substance that may represent serum proteins This was also found between epithelial cells and in the posterior chamber (Fig 1B)

In the iridial processes and in the anterior parts of the ciliary processes prominent vascular changes were observed Frequently large gaps between endothelial cells were found (Fig 4) sometimes with concurrent rupture of the

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Fig 2

Leakage of peroxidase (HRP) through intercellular spaces of the superficial ciliary epithelium A Epithelium of iridial process PGE_1 The tracer fills the lateral intercellular space (arrows) between superficial epithelial cells (SE) from the dilated ciliary channel (CC) to the posterior chamber (PC) The internal limiting membrane (ilm) has been stained by tracer material Uncontrasted section × 61 500 B Epithelium of anterior part of ciliary process PGE_2 HRP is seen as continuous electron dense lines throughout lateral intercellular spaces (arrows) of the superficial epithelium Uncontrasted section × 7 900

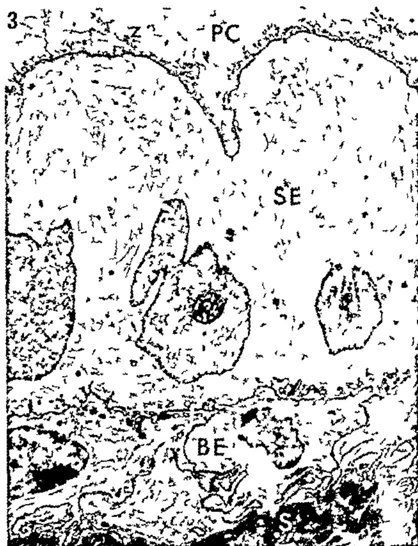


Fig 3

Posterior part of ciliary process PGF$_1$ From the same eye as Fig 2A HRP is seen in the stroma (S) between the basal epithelial cells (BE) and in the intercellular space between the basal and the superficial (SE) epithelial cells The HRP distribution and the structure of the epithelium are indistinguishable from that found in the control eyes Posterior chamber (PC) Zonular fibrils (z) Uranyl and lead × 5 600

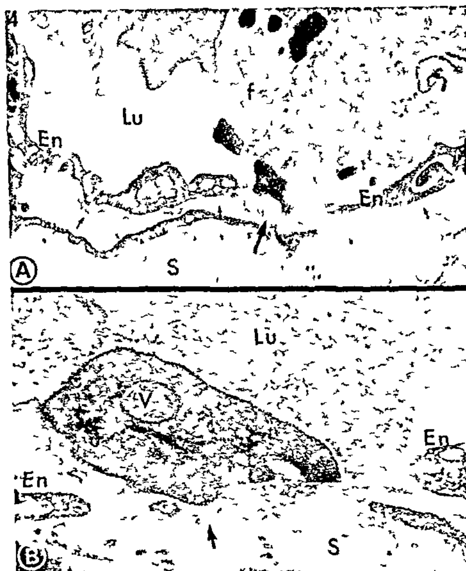


Fig 4

Ciliary vessels Iridial processes PGE. A Defect in the vascular endothelial lining (big arrow) The basement lamina (small arrows) of the endothelium (En) is not present in the defect In the vascular lumen (Lu) degranulated platelets and a fibrin like material (f) are seen Stroma (S) Uranyl and lead ×2 400 B Large gap between endothelial cells (En) A degranulated platelet is seen sticking to the basement lamina (arrow) In the vascular lumen (Lu) a fibrin like material surrounds the platelet A vacuole (V) in the platelet contains the same material Uranyl and lead ×47 000

5

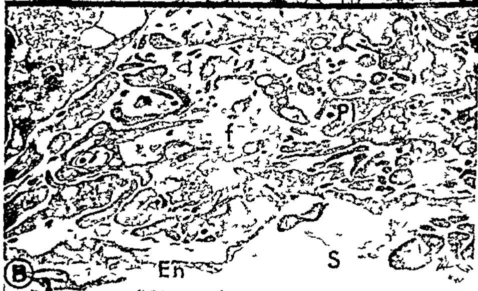
Pl

Lu

En

S

A



basement lamina In such defects of the endothelium degranulated platelets entangled in a fibrin like meshwork, could be demonstrated (Fig 4B)

Some vessels appeared congested with packing of erythrocytes in the lumen Moreover platelet aggregates were observed (Fig 5) Erythrocytes and platelets in the process of leaving the vessel were occasionally observed and numerous degranulated platelets (ghost cells) were present in the stroma (Fig 5A) In some vessels microthrombi were observed (Fig 5B)

Discussion

After topical application of PGE_1 and E_2 to rabbit eyes in the present study the following pathological changes have been demonstrated in the iridial and ciliary processes

1 Leakage of intravenously injected HRP through the intercellular spaces of the epithelium

2 Morphological changes in the epithelium of the iridial processes

3 Vascular changes including opening of interendothelial gaps rupture of the basement lamina haemorrhages platelet aggregations and formation of microthrombi

In a previous *in vitro* study on the effects of PGE_1 E and F_2 on the movement of HRP in the ciliary epithelium (Pedersen & Tønjum 1975) no leakage of the tracer through the ciliary epithelium was found and the *onulae occludentes* were found to be intact Moreover no significant morphological changes of the epithelium were observed These observations indicate that when the hydrostatic pressure gradient across the epithelium is zero PGs do not have a *direct* effect on the protein permeability of the *onulae occludentes* of the ciliary epithelium By *in vivo* application of PGs on the other hand the epithelial diffusion barrier to HRP is broken as recently demonstrated by Vegge Neufeld & Sears (1975) and in the present work Possibly the PGs

Fig 5

A Vessel in iridial process PGE_1 Accumulation of platelets (Pl) in the vascular lumen (Lu) A platelet is leaving the vessel (big arrow) Numerous degranulated platelets (ghost cells) (small arrows) are seen in the stroma (S) Endothelium (En) Uranyl and lead × 14 800 B Microthrombus in ciliary vessel PGE_2 In the vessel lumen, degranulated platelets (Pl) are found embedded in a fibrin like material (f) Uranyl and lead × 8 900

exert their effects on the epithelium via effects on the vessels Supporting this hypothesis marked vascular changes were found in those regions of the processes that displayed leakage of HRP through the intercellular spaces of the epithelium

The morphological changes of the epithelium of the iridial processes are in essence in accordance with observations made by Tamura (1974) who studied morphological changes in the rabbit ciliary body following topical application of PGE_1 and E_2

The epithelial changes also have striking similarities to alterations of the epithelium found in eyes after paracentesis of the anterior chamber (Smelser & Pei 1965 Bairati & Orzalesi 1966 Kozart 1968) and in experimental immunogenic uveitis (Segawa & Smelser 1969 Pedersen 1974) Evidence indicating that PGs are involved in these reactions has been presented (Eakins et al 1972 Neufeld Jampol & Sears 1972)

As far as the author is aware the participitation of platelets in the ocular reaction to PGs has not been reported before Tamura (1974) and Cole (1974) have made observations indicating that the permeability of the ciliary vessels is increased Thus Tamura (1974) found haemorrhages in the processes as well as leakage of intravascularly injected thorotrast from these vessels following conjunctival application of PGE_1 and E_2 and Cole (1974) demonstrated carbon labelling of the vessels following intracameral administration of PGE Electron microscopy was not performed by Cole (1974) but the study indicates that defects of the vascular endothelium had formed The endothelial defects demonstrated in the present study would allow these vessels to be labelled with carbon particles and may represent the morphological substrate for this phenomenon

Concerning the vascular changes observed in the present study there may be two possible sequences of events The platelet reaction may be secondary to injuries of the vascular endothelium due to vasodilatory effects of PGs (for references see Horton 1969) By the opening of interendothelial gaps and exposure of the basement lamina to the vessel lumen the platelet reaction may be triggered On the other hand it can not be excluded that the platelet reaction is the prime event initiating the inflammatory reaction By degranulation of platelets vasoactive substances and lysosomal enzymes are released

PGE_1 and E_2 have pronounced effects on platelet functions in various species (Chandrasekhar 1967 Emmons et al 1967 Kloeze 1967 Berman et al 1969) Generally PGE_1 inhibits the ADP induced platelet aggregation while PGE has the same effect in some species and increases the aggregation in others Whether direct effects of PGs on platelets are involved in the reaction studied in the present work can not be answered on the basis of the present observations

The present study on the ocular effects of PGs *in vivo* and previous observa

tions on the ciliary epithelium *in vitro* (Pedersen & Tønjum 1975) indicates that PGs may disrupt the ciliary epithelial diffusion barrier to proteins via effects on the vessels. Alterations in the hydrodynamics of the processes may be of importance in this connection. The observed oedema of the processes in the PG treated eyes indicates that the hydrostatic pressure in the stroma is increased. This may weaken the stability of the occluding zonules. Degranulation of platelets with release of vasoactive substances and lysosomal enzymes may also be of importance in this process.

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ELECTRON MICROSCOPIC STUDIES ON THE BLOOD AQUEOUS BARRIER OF PROSTAGLANDIN TREATED RABBIT EYES

II Iris

BY

OLAV ØYVIND PEDERSEN

The distribution of intravenously injected horseradish peroxidase in the iris of prostaglandin (E_1 and E_2) treated rabbit eyes has been studied with the electron microscope Peroxidase was demonstrated in the stroma of all parts of the iris The tracer was found throughout interendothelial clefts of iris vessels indicating that these vessels had become permeable to peroxidase The distribution of peroxidase in the iris epithelium indicates that the posterior epithelial cells are girdled by *zonulae occludentes*

Key words prostaglandins – iris – blood vessels – aqueous humour – anterior chamber – proteins – peroxidase – microscopy electron – rabbits

In the preceding work (Pedersen 1975) structures related to the blood aqueous barrier in the iridial and ciliary processes of prostaglandin (PG) treated rabbit eyes have been studied with the electron microscope

In the present paper observations on the iris from the same eyes are presented

Presented in part at the Third Meeting of the European Club for Ophthalmic Fine Structure March 7th 1975 Marburg West Germany

Material and Methods

Iris tissue was obtained from the same eyes used in the preceding work (Pederse 1975) The reader is referred to this paper for details concerning the materi and methods In the present work the gross distribution of horseradish peroxi dase (HRP) in the anterior uvea was studied in meridional frozen section mounted on glass slides after incubation in diaminobenzidine H O solution Central as well as peripheral parts of the iris including vessels and the anterio and posterior surfaces were studied with the electron microscope

Results

In the following the abbreviation HRP is used interchangeably for horse radish peroxidase and its reaction product

1 Control eyes

The gross distribution of HRP in the anterior uvea was studied in frozen sections The iridial and ciliary processes were blackened by HRP whereas in the iris and ciliary body base the HRP was confined to the vessel lumina (Fig 1B) These findings are in agreement with previous observations (Pederser & Tonjum 1975a)

Electron microscopy showed no leakage of HRP from the iris vessels The lumina of these vessels contained HRP but this was not found outside the endothelium i e in the basement lamina or in the iris stroma (Fig 3B) These observations are consistent with previous studies on iris vessels in which HRP was used as a tracer on the electron microscopic level (Shiose 1971 Smith 1971 Vegge 1971 Uusitalo Palkama & Stjernschantz 1973 Pedersen 1974b)

2. PG treated eyes

The observations were identical in all PG treated eyes irrespective of whether PGF_1 or E_2 was used

The gross distribution of HRP as revealed in the frozen sections from HRP injected animals was different from that found in the control eyes (compare Figs 1A and B) Apart from the processes and the vessels the stroma of the iris and the ciliary body base was blackened by HRP (Fig 1A) Prominent HRP staining was also observed in the pericorneal region (Fig 1A)

The presence of HRP in the iris stroma was confirmed by electron microscopy

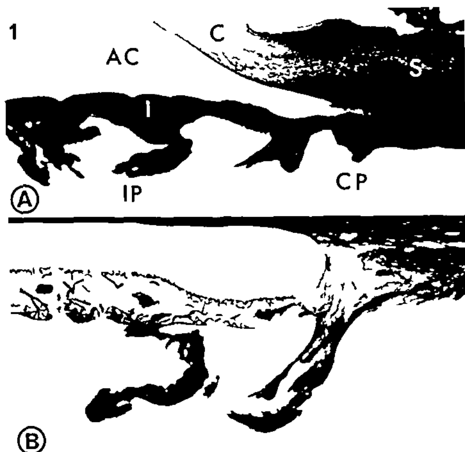


Fig 1

Meridional frozen sections cut through the anterior segment of rabbit eyes Dark staining is due to content of HRP in the tissues A PGE_1 Heavy staining is present in the iridial (IP) and ciliary (CP) processes as well as in the iris stroma (I) Anterior chamber (AC) Cornea (C) Sclera (S) ×40 B Control eye In the iris HRP is confined to the vessels ×40

HRP was found between the smooth muscle cells of the *sphincter pupillae* (Fig 2B) beneath the epithelium (Fig 4) between the endothelial cells at the anterior surface (Fig 2A) and adhering to collagen fibrils in all parts of the iris

In some places HRP could be followed continuously throughout the inter endothelial gaps of the vessels (Fig 3A) This phenomenon was observed in the

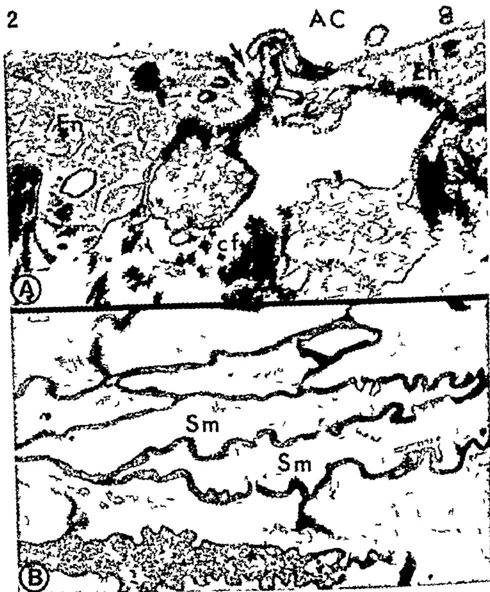


Fig 2

A Anterior surface of iris HRP is present on the surface of the endothelial cells (En) at some places between the endothelial cells (arrow) and adhering to collagen fibrils (cf) in the stroma Anterior chamber (AC) Uranyl and lead × 22 000 B HPP is present in the intercellular spaces between smooth muscle cells (Sm) of the *sphincter pupillae* Uncontrasted section × 7 400

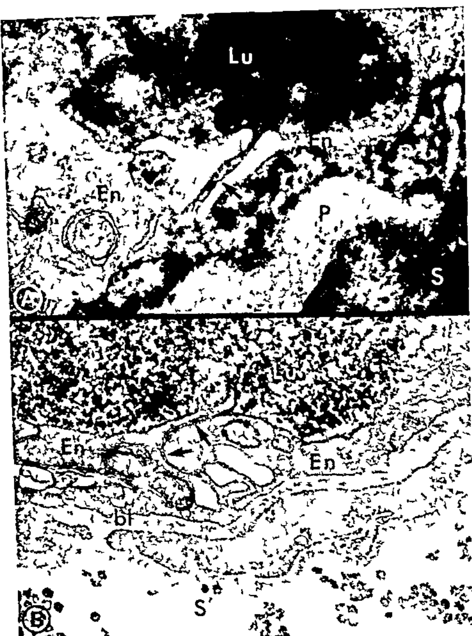


Fig 3

Iris vessels A PGE$_1$ HRP is present in the vascular lumen (Lu) throughout the interendothelial cleft (arrow) and in the stroma (S) Endothelial cells (En) Pericyte (P) Uranyl and lead. × 58 400 B Control eye. HRP is present in the vascular lumen (Lu) and in the luminal part of the interendothelial cleft The rest of the interendothelial cleft (arrows) is free of tracer material as are the basement lamina (bl) and the stroma (S) Uranyl and lead × 36 000

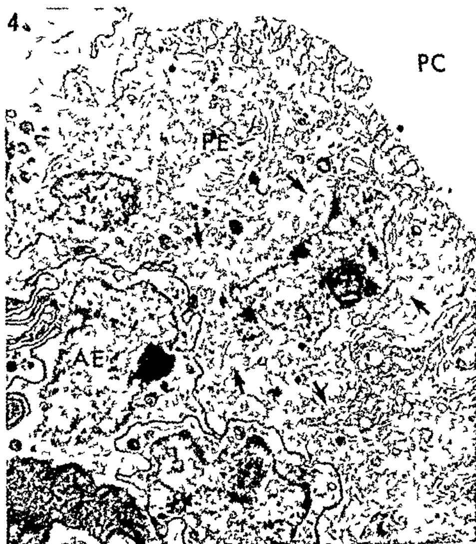


Fig 4

Iris epithelium PGE_1 HRP is present in the stroma (S) beneath the epithelium as well as in the intercellular spaces between the anterior epithelial cells (AE) and in the intercellular space between the anterior and posterior (PE) epithelial cells The movement of HRP towards the posterior chamber has been blocked at the basis (the stromal side) of the posterior epithelium and the lateral intercellular spaces (arrows) of the posterior epithelium do not contain tracer material HRP is seen in some vesicles in the epithelial cells Uranyl and lead × 9,500

small calibred vessels as well as in vessels with larger diameters As in the control eyes HRP containing endothelial vesicles were few in number

Widely opened interendothelial gaps were not observed in the iris vessels and no haemorrhages or platelet aggregations were present

In the iris epithelium the tracer had entered the lateral intercellular spaces of the anterior cell layer as well as the intercellular space between the anterior and the posterior epithelial cells The movement of HRP from the iris stroma towards the posterior chamber had come to an abrupt stop at the basis (the stromal side) of the posterior epithelial cells and the lateral intercellular spaces between these cells were usually free of tracer material (Fig 4) In some places the internal limiting membrane covering the epithelium contained HRP which apparently had spread from leakage sites in the ciliary epithelium (Pedersen 1975)

The observations on the HRP distribution in the iris epithelium indicate that the posterior epithelial cells of the iris like the superficial (non pigmented) epithelial cells of the ciliary processes are girdled by *onulae occludentes* This finding is consistent with previous observations on the iris epithelium *in vitro* in which HRP was used as an electron microscopical tracer (Pedersen & Tonjum 1975b)

Discussion

When HRP is introduced into the bloodstream of the rabbit as a microscopical tracer a characteristic distribution pattern of the tracer is found in the anterior uvea (Pedersen & Tonjum 1975a) The present study has shown that when PGE_1 or E_2 is applied topically to the rabbit eye before the HRP injection a pronounced alteration of this distribution pattern is found Thus in the iris heavy amounts of the tracer are present in the stroma Several possibilities exist concerning the source of HRP found in the iris stroma 1 Leakage of HRP through the interendothelial clefts of iris vessels 2 Vesicular transport of HRP through the endothelium of iris vessels 3 Anterior movement of HRP from the stroma of the iridial processes into the iris stroma 4 Diffusion of HRP present in the aqueous humour into the iris stroma via the anterior iris surface

In the present study evidence of leakage from the iris vessels has been presented Thus in some places HRP could be followed throughout the interendothelial clefts of iris vessels

There is no indication that vesicular transport of HRP across the endothelium of the vessels is of significance as HRP containing vesicles did not appear to be more frequent in the PG treated eyes as compared with the controls

The possibility that HRP may move anteriorly from the iridial processes into the iris stroma can not be ruled out In the frozen sections a continuous blackening from the stroma of the processes to the anterior surface of iris was observed This picture however can be explained solely on the basis of increased permeability of the iris vessels to HRP

By leakage of HRP through the ciliary epithelium (Pedersen 1975) the concentration of the tracer in the aqueous humour will increase during the period from the HRP injection to the enucleation However, diffusion of HRP from the aqueous humour into the iris stroma via the anterior surface of iris seems to be of minor significance Thus HRP staining of structures lining the anterior and posterior chambers was not found constantly and the amount of reaction product observed in the anterior parts of iris was not greater than in other parts of this organ Moreover in electron micrographs published by Raviola (1974) who studied the effects of paracentesis on the blood aqueous barrier in monkeys using HRP as a tracer no reaction product can be seen in the iris stroma although large doses of HRP (500 mg per kg body weight) was injected intravenously and the aqueous humour became discoloured by the tracer

One might argue that HRP found in the interendothelial clefts of the vessels on the stromal side of the junction came from the abluminal side by diffusion from the processes as no clear evidence of disruption of the junctions was found However this is not likely because the tracer was demonstrated throughout the interendothelial clefts also in the region close to the pupillary margin which is not covered with processes

On the basis of the observations in the present study it is concluded that the iris vessels become permeable to the protein tracer HRP when PGE_1 or E_2 is applied topically to the rabbit eye The possibility that HRP may diffuse anteriorly from the stroma of the iridial processes through the iris in such eyes can not be ruled out

Widely opened interendothelial gaps in the iris vessels were not found in the present material This is consistent with observations made by Cole (1974) After intracameral perfusion of rabbit eyes with PGE no carbon labelling of iris vessels was found This observation is not contradictory to the HRP leakage observed in the present study as the diameter of the carbon particles are about 10 times that of the peroxidase molecules Observations indicating increased permeability of the iris vessels following application of PGs have been presented by Whitelocke & Eakins (1973) These authors found leakage of fluorescein from iris vessels *in vivo* after conjunctival application of PGF_1 F and F to rabbit eyes

The relatively sparse pathological findings in the iris vessels in the PG treated eyes are in contrast to the marked vascular changes found in the vessels

of the iridial and ciliary processes described in the preceding paper (Pedersen 1975) This may be due to differences in structure of these vessels The iris vessels have a thick endothelium without *fenestrae* and the endothelial cells are girdled by *onulae occludentes* while the vessels of the processes have a very thin endothelium containing *fenestrae* and the attachments between the endo thelial cells are insecure *Zonulae occludentes* are lacking

The observations on the HRP distribution in the iris epithelium indicate that the posterior epithelial cells are girdled by *onulae occludentes* as this is the only known type of junction that effectively blocks movement of HRP through the intercellular spaces of epithelia The functional significance of this observa tion is not clear *Zonulae occludentes* restricts not only the movement of high molecular watersoluble substances but also to a variable degree the movement of water and ions Hence the iris epithelium represents a barrier against move ment of aqueous humour from the posterior to the anterior chamber From the posterior chamber the aqueous humour is thus forced through the pupil to the anterior chamber This may have some significance as it prevents undue scrub bing of the iris on the anterior lens surface The demonstration of *onulae occlu dentes* in the iris epithelium explains the development of *iris bombee* due to *seclusio pupillae* and the clinical observation that when iridectomy is performed to release this condition removal of the epithelium is essential

PGE_1 and E_2 when applied topically to the rabbit eye produce marked alterations of structures related to the blood aqueous barrier Leakage of the protein tracer HRI across the barrier is found in the ciliary epithelium (Peder sen 1975) and in iris vessels Thus these PGs induce some of the changes found in experimental immunogenic uveitis (Pedersen 1973 74a 74b) Several observa tions indicate that PGs may be involved in this reaction Increased amounts of PGs have been detected in the aqueous humour of rabbits with this type of uveitis (Eakins et al 1972a) and indomethacin known to depress PG synthesis (Vane 1971) has anti inflammatory effects against the condition (Hanna & Keatts 1967)

PGs may be involved in acute anterior uveitis (iridocyclitis) in the human Thus E and F PG like activity has been detected in the aqueous humour of patients with untreated acute anterior uveitis (Eakins et al 1972b) In an early double blind study on uveitis in man a greater number of improvements were found in the group treated with indomethacin mydriatics and steroids than in the group where indomethacin was excluded (Perkins & MacFaul 1965) As mentioned above indomethacin and also salicylates inhibit PG synthesis (Vane 1971)

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EFFECTS OF ETHYL ALCOHOL ON THE DIRECTLY RECORDED STANDING POTENTIAL OF THE HUMAN EYE

BY

KLAS OLAV SKOOG OLA TEXTORIUS
and SVEN ERIK G NILSSON

The effects of ethanol on the human standing potential (SP) were studied with a recently developed method which allows direct SP recordings by mean of a suction contact lens temperature stabilized calomel electrodes and d c amplification It is well known that the human SP oscillates with a frequency of about ?/hour in response to a sudden change in illumina tion In the present paper marked cyclic variations of the SP resembl ng damped oscillations were provoked by a small oral dose of ethyl alcohol A first maximum was reached after about 10 min The difference in amplitude between the peak and the trough of the first oscillation was of the order of 4 mV The oscillatory frequency was about 2/hour The length of a cycle varied between 25 and 34 min in different volunteers being fairly constant in the same subject on different occasions The SP response to ethanol was similar both under scotopic and photopic condi tions The results correlate well with earlier findings of 2/hour oscillations in c wave amplitude in response to ethanol as may be expected considering the partly common origin of the c wave and the SP

Key words ocular electrophysiology – standing potential – ethyl alcohol – retina – pigment epithelium

The so called standing potential (SP) between the anterior and posterior pole of the eye the cornea being positive to the fundus in vertebrates was dis covered by du Bois Reymond in 1849 (frog)

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More or less accurate direct SP recordings from animals were performed by Yoshida (1953) (toad) Muller Limmroth (1954) (frog) Kikawada (1968) (*pisces amphibia reptilia aves rodentia*) Very stable direct registrations were published by Knave Persson & Nilsson (1974a b) (sheep)

Until recently because of technical difficulties it has been necessary to use an indirect method (electrooculogram EOG) to estimate the human SP (François Verriest & De Rouck 1955 Arden Barrada & Kelsey 1962 and Arden & Kelsey 1962) A newly developed method (Skoog 1975) including a suction contact lens and matched calomel half cell electrodes permitted direct recordings of the human SP under stable conditions With a similar technique stable and reproducible registrations of the *c* wave of the human d c electro retinogram (ERG) were obtained (Knave Nilsson & Lunt 1973 Skoog & Nilsson 1974a b)

An increasing amount of research has been devoted to the pigment epithelium because of its vital importance to the outer retinal layers In this connection it is interesting to note tha both the *c* wave of the ERG (Noell 1954 Brown & Wiesel 1961 Steinberg Schmidt & Brown 1970 and Schmidt & Steinberg 1971) and the SP (Noell 1954 Heck & Papst 1957 and Gouras 1969) are known to be generated mainly in the pigment epithelium The *c* wave is suggested to originate as a result of hyperpolarization of pigment epithelial cells in response to a decrease in potassium ion concentration around photoreceptors upon light stimulation (Miller & Steinberg 1975 Oakley & Green 1975) Thus both variables reflect pigment epithelial activity although presumably in somewhat different ways

Animal experiments have demonstrated effects on the *c* wave and SP by various drugs some of which are known to show affinity to the pigment epithelium

Changes in *c* wave amplitude were observed in response to epinephrine (Therman 1938) sodium azide and sodium iodate (Noell 1954) the anti tuberculous drug rifampicin (Knave Persson Calissendorff & Nilsson 1973) barbiturates (Knave Persson & Nilsson 1974a b) chlorpromazine and chloroquine (Calissendorff 1975)

Strychnine and urea diminished the SP according to Muller Limmroth & Lemaitre (1953) The SP was dramatically decreased by the injection of sodium iodate while sodium azide caused an increase (Noell 1954 and Heck & Papst 1957) Small doses of barbiturates provoked a negative shift in the SP while larger doses started a negative – positive cyclic variation of the SP resembling damped oscillations (Knave Persson & Nilsson 1974a b)

The effects of ethanol on the *a* and *b* waves of the human ERG were extensively studied by Straub (1957) Ikeda (1963) Jacobson Hirose & Stokes (1969)

and in rabbits by Manfredini & Trimarchi (1968) and Morita (1970). Comparatively few reports have been published about ethanol action on the *c* wave and the SP. In experiments on sheep Knave, Persson & Nilsson (1974a) found a large increase in *c* wave amplitude after i.v. ethanol injection. In other registrations by the same authors the effects of i.v. ethyl alcohol on the SP were studied: after a small negative shift there was a large positive change of long duration.

The effect of ethanol on the human *c* wave was investigated (Skoog 1974) with the aid of the method described by Knave, Nilsson & Lunt (1973) and by Skoog & Nilsson (1974a,b). The *c* wave is known to respond to repeated light flashes with a slow cyclic amplitude oscillation (frequency about 2/hour) (Skoog & Nilsson 1974b (man) and Calissendorff, Knave & Persson 1974 (sheep)). A small oral dose of ethanol elevated the mean level of these oscillations and also caused a marked increase in their amplitude. The first maximum occurred 10–15 min after the ingestion of alcohol (Skoog 1974).

To our knowledge there has been no previous report on the effect of ethyl alcohol on the directly recorded human SP. Considering the close relationship between the *c* wave and the SP it seems desirable to carry out such recordings. Like the *c* wave the SP is known to oscillate in response to a change in light conditions as demonstrated in direct SP recordings (Skoog 1975) and with the aid of EOG (Kris 1958, Kolder 1959, Taumer, Hennig & Pernice 1974 and others). It may be hypothesized that this oscillatory behaviour is a stereotype form of SP response to a variety of stimuli, as in the case of the *c* wave oscillations. In the present study ethyl alcohol was chosen as a test substance since its absorption and other pharmacological properties are well known (Goldberg 1943, Haggard, Greenberg & Lolli 1941).

Material and Methods

The present recording technique was described by Skoog (1975).

Five healthy female volunteers, aged 22–31 years and weighing 56–70 kg, participated in the study. Tropicamide (0.5 %) and metaoxedrine (10 %) given topically dilated the pupils to 8 mm or more. After topical anaesthesia a scleral contact lens was applied to one of the eyes (Fig. 1). Two polyethylene tubes were attached to the lens. One, filled with saline, ended in a small beaker of saline, the surface of which was located 20 cm below the level of the eye. This arrangement produced a slight suction which kept the lens still in relation to the eye. The other tube was a saline – agar bridge leading to the recording electrode. Similar bridges connected the reference and ground electrodes to



Fig 1

The contact lens and the two chambers attached to a volunteer Saline bridges in polyethylene tubes lead to the calomel half cells (left) Two of these are temperature stabilized and used as recording and reference electrodes respectively The third electrode mediates the ground connection.

plastic chambers placed on the forehead above the examined eye and above the lateral canthus of the other eye respectively (Fig 1) The contact lens as well as the chambers were filled with Methocel® Significant changes in intraocular pressure were excluded through applanation tonometry before the application and immediately after the removal of the lens The absence of corneal abrasions after the experiments was checked for with the aid of slit lamp examination and fluorescein staining

The signals from the eye were picked up by temperature stabilized (± 0 1 C) matched calomel half cells (Fig 1) and then reached a low drift d.c amplifier Also the ground connection was mediated by a calomel electrode A wire net cage protected the electrodes and the volunteer from electrical disturbances After lowpass filtering (20 Hz cut off 18 dB/octave) the signals passed from the amplifier to a Hewlett Packard 7402 A ink writing recorder where the SP variations were traced During registrations the volunteers fixated a very weak deep red light in the ceiling One subject was able to keep her eyes quite still throughout the experiments and in her case continuous stable recordings were obtained without fixation

After the application of the electrodes the subjects were left for about 75 min either in darkness or in a constant illumination of the eyes of 1100 Lux. In this way the SP finally stabilized to a base line. The average potential during 10 min before the ingestion of ethanol is referred to as zero potential. If fixation was needed, recordings were carried out twice a minute.

0.4 g ethanol/kg body weight was given orally in the form of brandy with an addition of 95 vol % ethanol to make 50 vol %. The dose was taken in less than one min, during which the electrodes were kept in place.

The volunteers were fasting for 4 hours prior to the intake of alcohol. Before that they had only a very light meal (e.g. a sandwich and a glass of juice). Volunteers were not under the influence of any previous intake of alcoholic beverages. The blood alcohol analyses (gas chromatography) were carried out at the Department of Clinical Chemistry.

In all, 12 experiments were performed.

Results

The oral administration of ethanol provoked long term cyclic variations in SP resembling damped oscillations with a frequency of about 2/hour. This was the case both under steady scotopic and photopic light conditions.

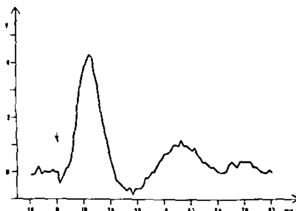


Fig. 2

The results of a typical experiment performed in darkness, showing SP oscillations provoked by the oral administration (arrow) of 0.4 g ethanol/kg body weight. SP recorded twice a minute. Each point on the curve represents the average of two consecutive measurements.



Fig 3

SP oscillations provoked by the oral intake (arrow) of 0 4 g ethanol/kg body weight Illumination of the eyes 1100 Lux Recording procedure and plotting as for Fig 2

Fig 2 illustrates the results from a typical experiment in darkness where 0 4 g ethanol/kg body weight was given orally A marked increase in SP was initiated reaching its maximum after 12 min This was followed by a trough at 28 min and a second smaller peak at 46 min after the beginning of alcohol ingestion The other experiments performed in darkness confirmed the illustrated findings The average amplitude difference between the peak and the trough of the first oscillation was of the order of 4 4 mV The average latency of the first peak was 10 5 min

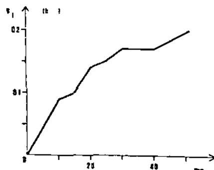


Fig 4

Blood alcohol levels in g/litre from the experiment illustrated in Fig 3
Alcohol was given at time 0

Similar oscillations as those obtained with alcohol in darkness were provoked by ethanol also in eyes adapted to an illumination of 1100 Lux as may be seen in Fig 3 The first peak appeared at 10 min the trough at 22 min and the second oscillatory maximum at 35 min The other experiments performed with an illumination of 1100 Lux confirmed the above findings The average latency of the first maximum was 10 min The amplitude of the first oscillation was 3 4 mV on an average

In some registrations the oscillations seemed to be superimposed upon a very small and much slower change but the duration of experiments was not long enough to define the exact nature of this variation

Fig 4 demonstrates the increase in blood alcohol concentration after the administration of 0 4 g ethanol/kg body weight in the experiment illustrated in Fig 3

The duration of one main oscillation varied between 25 and 34 min in the different registrations seeming to be fairly constant for a certain volunteer on different occasions

Discussion

The present study clearly demonstrated that a single small oral dose of ethanol provoked dramatic and long term effects on the SP in the form of damped 2/hour oscillations Because of the low noise level and the stability of the recording system it was possible to trace SP reactions even after 60 min

In some registrations the 2/hour oscillations seemed to be superimposed upon a much slower and smaller change The exact nature of the latter cannot be determined on the basis of the present study For this reason even more prolonged experiments are already in progress The slow variation may correspond to the 0 5/hour c wave oscillations demonstrated by Calissendorff Knave & Persson (1974) (sheep) Also a minor change might be attributed to variations in properties of the skin – electrode junction (e g increased sweating) induced by alcohol Through fluorescein staining of cornea and applanation tonometry directly after the recording corneal abrasions and significant intraocular pressure changes which might have influenced the results were excluded The d c drift of the electrode system continuously followed for 1 hour did not exceed 15 μV After the attachment of the electrodes the described background illumination was adjusted This change in light conditions of course started more or less pronounced SP oscillations Thus recordings were not begun until the oscillations had been damped and the base line was stable

Repeated venous blood samples revealed that the maximum SP amplitude occurred while blood alcohol concentrations were still increasing The same was true for the *c* wave maximum in similar experiments (Skoog 1974) It has been shown in earlier psychophysical experiments that a certain blood alcohol level exerts a greater influence on test parameters during rising than during diminishing concentrations (Goldberg 1943 and others)

Knave Persson & Nilsson (1974a) (sheep) injected *i v* ethanol and obtained a slow positive peak in SP resembling the beginning of the oscillations of the present study but they also noticed an initial negative transient of less than 2 min duration The oral route of administration used in the present study was probably too slow to demonstrate clearly any initial fast changes The above authors reported an amplitude maximum about 8 min after the *i v* injection while in the present study this peak occurred somewhat later This difference most likely depends on the different modes of administration

The observed SP variations in response to ethanol correlate well with those of the *c* wave after corresponding doses The latency of the first maximum as well as the frequency of the oscillations were of the same magnitude in both types of experiments This similarity in behaviour is not surprising considering the partly common origin of the *c* wave and the SP (see above) In this connection it is interesting to note that the alcohol response of the SP was the same under scotopic as well as under photopic conditions It thus seems that these oscillations of the SP are not dependent on the level of rod sensitivity to light The *c* wave is proposed to be rod dependent (Steinberg Schmidt & Brown 1970 and others) In experiments now in progress no *c* waves could be demonstrated at a photopic level of adaptation at which ethanol induced SP oscillations were easily elicited

It is not possible to explain the observed ethanol effects only on the basis of the present investigation According to Knutsson (1961) Knutsson & Katz (1967) Inoue & Frank (1967) Houck (1969) and Bergmann Klee & Faber (1974) the cell membrane potential is altered by ethanol Of course both the pigment epithelial cells and the retinal cells could be affected in this manner and Knave Persson & Nilsson (1974a) suggested a dual site of action of ethanol The *c* wave and SP variations provoked by ethanol are very similar to the well known light induced oscillations of both variables It seems likely that this cyclic behaviour is a stereotype way of reacting to various stimuli It might be of interest to try to elicit oscillations with other kinds of stimuli such as different drugs and substances not only to study possible toxic drug reactions but also as an attempt to clarify the exact origin of the oscillations

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COVARIATION OF THE SIMULTANEOUSLY RECORDED c WAVE AND STANDING POTENTIAL OF THE HUMAN EYE

BY

SVEN ERIK G NILSSON and KLAS OLAV SKOOG

The *c* wave and the directly recorded standing potential (SP) of the human eye were studied with the aid of a recently developed method including matched temperature stabilized calomel electrodes d c amplifiers and a suction contact lens This technique which does not require general anaesthesia permits simultaneous direct d.c recordings of the SP and the *c* wave in human volunteers during long term experiments Upon repetitive light flashes (stimulus duration 1 sec interval 90 sec and flash intensity 4 5 rel log units above *b* wave threshold) both variables responded with slow amplitude oscillations with a frequency of about 2/hour The oscillations were similar as to phases and frequencies Both the potentials are held to be generated mainly in the pigment epithelium Considering this partly common origin the observed covariation was an interesting finding

Key words ocular electrophysiology – electroretinography – *c* wave standing potential – retina – pigment epithelium

Since the foundations of clinical electroretinography (ERG) were laid independently by Riggs (1941) and Karpe (1945) mainly the comparatively fast *a* and *b* waves and their subcomponents have been extensively studied This was possible with the aid of rather uncomplicated silver – silver chloride elec

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trodes and condenser coupled (a c) amplifiers However neither of these systems permit true registrations of the c wave and other slow potentials which instead require direct coupled (d c) amplifiers and very stable electrodes Probably because of technical difficulties or for other reasons very little has been published on the human c wave Dodt (1951) working with Ag – Ag Cl electrodes and Wirth (1951) using a c amplification were able to demonstrate human c waves which tended to disappear after repeated light flashes With the aid of general anaesthesia human c wave recordings were obtained by Hanitzsch Hommer & Bornschein (1966) and Heilig Thaler & Bornschein (1973)

While investigating the c wave of the human d c registered ERG Knave Nilsson & Lunt (1973) Skoog & Nilsson (1974a b) and Skoog (1974) obtained very stable recordings by means of special calomel electrodes but without the aid of general anaesthesia The c wave amplitude was shown to be linearly related to log stimulus light intensity within the range studied (2 log units) (Skoog & Nilsson 1974a) Damped cyclic variations of the c wave amplitude were found in response to repetitive light flashes in long term experiments The frequency of the oscillations was about 2/hour (Skoog & Nilsson 1974b) The same kind of variation was also provoked by a small oral dose of ethyl alcohol (Skoog 1974) The above results are in agreement with findings from similar experiments on sheep (Calissendorff Knave & Persson 1974 Knave Persson & Nilsson 1974a)

The addition to the method of a suction contact lens electrode further improved stability and it became possible to follow directly even the standing potential (SP) of the human eye without general anaesthesia for long periods of time (Skoog 1975) Earlier it was necessary to study the SP by an indirect method the electrooculogram (EOG)

In recent years an increasing interest has been focussed on the pigment epithelium which acts as a metabolically very active intermediate between the choroidal vascular bed and the neuroretina The pigment epithelium is involved in photoreceptor nutrition and photopigment regeneration (Hubbard & Wald 1951) and it also provides the phagocytic mechanism necessary for the continuous renewal of the photoreceptor outer segments (Young & Bok 1969) It is well known that drugs like certain phenthiazines and chloroquine (Potts 1962 Meier Ruge 1965 and others) may cause serious pigment epithelial injuries which may secondarily damage the neuroretina In this connection it is interesting to note that both the c wave and the SP are generated mainly in the pigment epithelium Both variables although probably in somewhat different ways reflect pigment epithelial activity under normal conditions and during the influence of diseases or drugs A fairly large pigment epithelial injury is needed before the EOG is affected It is hoped that careful studies of the c wave and

the directly recorded SP both separately and in simultaneous recordings might further elucidate pigment epithelial functions The aim is among other things to find a variable which will signal early pigment epithelial damage First however the characteristics of the *c* wave and the SP must be evaluated and compared under standardized conditions in normal volunteers

The direct method for following the SP was developed partly to serve in such simultaneous recordings of the *c* wave and the SP Since *c* wave registrations require very stable conditions it seems unrealistic to combine them with the EOG procedure On the other hand with the addition of a light source and some electronic modifications as described below the direct SP method easily yields also *c* wave recordings One principle characteristic shared by the *c* wave and the SP is their tendency to respond to stimuli with slow damped regular amplitude oscillations

The present paper demonstrates parallel 2/hour oscillations in SP and *c* wave amplitudes

Material and Methods

With some modifications the method is based on a combination of recently published techniques for d c *c* wave registrations (Knave Nilsson & Lunt 1973 Skoog & Nilsson 1974a b Skoog 1974) and for direct recordings of the standing potential (Skoog 1975) from human eyes

Five healthy volunteers females aged between 22 and 31 years were chosen They were not under the influence of drugs and other stimulants Local instillations of 0 5 % tropicamide and 10 % metaoxedrine dilated the pupils to 8 mm or more A scleral contact lens was applied to one of the eyes (Fig 1) It was equipped with two polyethylene tubes One was a saline agar bridge to the recording electrode and the other one filled with saline ended in a beaker of saline The surface of the latter was located 20 cm below the level of the eye and thus a gentle suction was obtained keeping the contact lens still in relation to the eye A plastic chamber (for the tip of the reference electrode) was placed on the forehead 4 cm above the cornea of the investigated eye (Fig 1) Another chamber (for the tip of the ground electrode) was located above the lateral canthus of the other eye The lens and the chambers were filled with Methocel® No significant changes in intraocular pressure were found through applanation tonometry before the application and directly after the removal of the contact lens At the same time corneal epithelial defects were excluded by means of fluorescein staining and slit lamp inspection

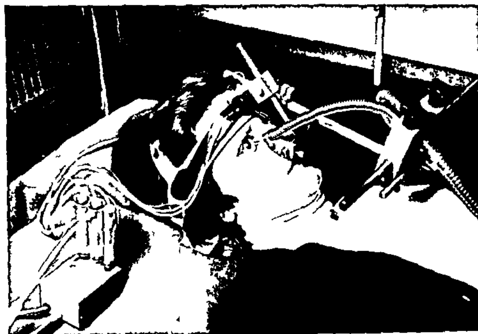


Fig 1

To the left the recording and reference calomel half cells temperature stabilized by means of circulating water The third calomel electrode mediates the ground connection. Saline bridges in polyethylene tubes pass from the recording reference and ground electrodes respectively to a suction contact lens a plastic chamber above the investigated eye and a second chamber above the lateral canthus of the contralateral eye

Matched calomel half cells temperature stabilized (± 0 1°C) with the aid of circulating water served as recording and reference electrodes (Fig 1) Also the ground connection was mediated by a calomel electrode Saline bridges in polyethylene tubes connected the two chambers and the contact lens with the calomel half cells A wire net cage protected the electrodes and the subject from external electrical disturbances The signals from the electrodes were fed into the differential inputs of a low drift d c amplifier with a very high impedance They were lowpass filtered (220 Hz cut off 18dB/octave) and then passed to a Tandberg analogue tape recorder and also reached a Hewlett Packard 5480 S signal analyser where the *c* wave recordings were displayed It was possible to balance the inputs of the analyser before each *c* wave registration so that a constant base line was maintained in spite of the expected slow SP variations The *c* wave amplitude was measured from this base line The signals from the d c amplifier also passed to a Hewlett Packard 7402 A ink writing recorder

which continuously displayed the SP and the timing of the *c* wave registrations Four *c* wave recordings were averaged but each of them could be inspected on a separate oscilloscope

Stimulus light was produced by a 150 Watt ozone free Osram XBO xenon lamp with an approximately flat spectral emission curve within the visible part of the spectrum It passed heat reflection and heat absorbing filters (Zeiss) Its intensity was kept at 4 5 rel log units above *b* wave threshold by means of neutral density filters (Balzer) The lowest intensity eliciting a single flash *b* wave (threshold at 30–40 μV) is referred to as log rel intensity 0 Stimulus duration was kept at 1 sec with an electromagnetic shutter (Zeiss) After the application of the electrodes the volunteers were left in total darkness for at least 75 min until the SP was constant During registrations each lasting 5 sec the subjects fixated a very weak deep red light in the ceiling The peak of the *c* wave could always be identified Recordings were repeated 3 times a minute The average SP level preceding the onset of flashing is referred to as zero potential Ten experiments were carried out each registration lasting 45–60 min

Results

Cyclic changes of the amplitudes of both the *c* wave and the SP of the human eye were produced by 1 sec light flashes at 20 sec intervals When these variables were simultaneously recorded a clear covariation was observed as demonstrated in Fig 2 This graph illustrates a typical experiment in which the SP varied like a damped oscillation Starting in a positive direction a maximum slowly appeared after 18–19 min and the variation continued with a second peak after 45 min Thus the oscillatory frequency was about 2/hour The amplitude difference between the peak and the trough of the first oscillation was 1 4 mV On repetitive stimulations also the *c* wave amplitude increased initially rea ching a peak of 460 μV which closely coincided in time with the first SP maximum Also the *c* wave amplitude oscillated with a frequency of about 2/hour The second maximum appeared at approximately the same time as the corresponding SP peak The amplitude difference between the maximum and the minimum of the first *c* wave oscillation was 270 μV

The above results were fully confirmed by the other registrations which were similar as to timing and magnitude of the variations In some experiments the 2/hour oscillations seemed to be superimposed upon a small and much slower change but the duration of each experiment was not long enough to demonstrate the exact character of this change

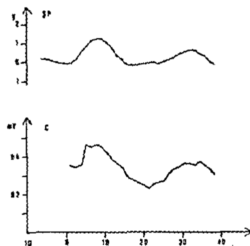


Fig 2

Simultaneously recorded cyclic changes of the SP level and the *c* wave amplitude measured from the base line in response to 1 sec light flashes repeated at 20 sec intervals Light intensity 4 5 rel log units above *b* wave threshold Each point on the curves represents the average of four measurements

Discussion

With the present recording system stable SP and *c* wave recordings were obtained during long term experiments Since the onset of dark adaptation provoked a cyclic change in SP it was necessary to wait until this oscillation had been damped before registrations were started After approximately 75 min the base line followed for about 10 min was stable enough One sec light flashes with a relative intensity of 4 5 log units above *b* wave threshold were chosen since they produced well defined *c* waves without causing immediate light adaptation of the *c* wave As was known from preliminary experiments a stimulus interval of 15 sec did not diminish subsequent *c* waves On the other hand it may be assumed that the observed parallel oscillations in SP and *c* wave amplitudes in response to repeated flashes are adaptation phenomena During flashing the average illumination of the retina exceeded that of the preceding dark adaptation Both the SP and *c* wave 2/hour oscillations started in a positive direction in response to the onset of stimulation This is in good agreement with the SP alteration after a sudden and lasting increase in illumination (and thus light adaptation) of the eye when after a short negative shift a 2/hour oscillation started in a positive direction (Skoog 1975) (Fig 3) Con

sidering the great similarities between the SP and *c* wave oscillations demonstrated in the present paper it may be suggested that they are provoked by common factors

In the present study the alteration in average illumination brought about by repeated flashing was probably not large enough to cause a detectable negative initial transient Alternatively the short interrupted flashes 3 min were for other reasons not adequate stimuli to elicit this fast change. Hennig Taumer & Pernice (1974) have shown that the fast oscillation is best provoked by sinusoidal light changes with a frequency of about 0 4/min.

In some recordings the 2/hour oscillations were superimposed upon a small and much slower change The experiments were too short to prove the nature of this variation but it could very well correspond to the 0.5/hour oscillations of the *c* wave found in experiments on sheep by Calissendorff Knave & Persson (1974) The possible existence of such 0.5/hour cyclic changes of the human *c* wave and SP are now being investigated in our laboratory It cannot be totally excluded that changes in properties of the body electrode junctions might slightly influence the recordings but variations in electrode impedance

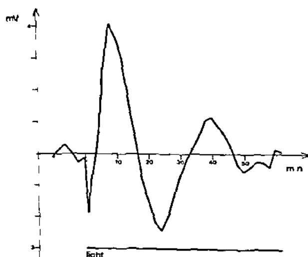


Fig 3
Directly recorded SP oscillations in response to a change in illumination from darkness to 16 Lux SP registered every min except from 0 to 5 min when 30 sec intervals were used Each point of the curve represents the average of two consecutive measurements (From Skoog 19 5)

would hardly influence the measurements considering the high input impedance of the amplifier Great care was taken not to injure the cornea or the skin during the application of electrodes No corneal damage and no significant change in intraocular pressure (IOP) was noted Stepanik (1958) needed considerably stronger suction to provoke IOP variations with a special contact lens *that was designed to block the aqueous outflow* The volunteers were kept awake throughout the long period of dark adaptation in order to avoid any possible effects on the SP of different levels of wakefulness as described in FOG experiments by Jacobs Feldman Rabinowitz & Bender (1973)

Since the pigment epithelium is considered to be a major source of the *c* wave (*Noell 1954 Brown & Wiesel 1961 Steinberg Schmidt & Brown 1970*) and of the SP (Noell 1954 Heck & Papst 1957 Gouras 1969) it is tempting to suppose that the 2 hour oscillations of both variables are produced in the pigment epithelium possibly through the interaction with receptors The *c* wave is rod dependent according to Steinberg Schmidt & Brown (1970) Miller & Steinberg (1975) found evidence that the *c* wave was produced by a decrease in the extracellular potassium ion concentration surrounding the apical membranes of the pigment epithelial cells Oakley & Green (1975) performed recordings with potassium specific microelectrodes and concluded that the *c* wave is produced by the pigment epithelial cells as they hyperpolarize in response to a decrease in potassium around the stimulated photoreceptors On the basis of experiments on diseased eyes Arden & Kelsey (1962) suggested that the oscillations of the FOG occurred in the pigment epithelium C Essendorff Knave & Persson (1974) proposed that the oscillations of the *c* wave in sheep could perhaps depend on changes in rod sensitivity In that connection it is tempting to believe that the fast and slow SP oscillations (Hennig Taumer & Pernice 1974 Taumer Hennig & Pernice 1974 and Skoog 1975) are derived through interaction between the pigment epithelium and cones and rods respectively the dark adaptation etc of the cones occurring considerably faster than that of the rods However it must be remembered that potentials from the neuroretina such as slow PIII may modify the shape of the *c* wave and that *the neuroretina influences the SP (Gouras 1969 and others) Therefore* it cannot be fully excluded that one or more of the oscillatory variations mentioned above might occur as a result of cyclic changes in the neuroretina Whatever the source of the 2 hour oscillations may be these seem to represent a stereotype way of reacting to different stimuli Barbiturates (Knave Persson & Nilsson 1974a b) (sheep) and ethanol (Skoog 1974 and Skoog Textorius & Nilsson 1975) (man) provoke such cyclic variations both in SP and in the *c* wave amplitude It is hoped that the present method for simultaneous recordings of these variables will be of value in comparative experiments with different drugs and substances

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MANAGEMENT OF TRAUMATIC RETINAL DETACHMENT WITH PARS PLANA VITRECTOMY SCLERAL BUCKLING AND GAS INJECTION

BY

GHOLAM A PEYMAN FELIPE U HUAMONTE

and

MARC ROSE

Vitreous involvement as a result of penetrating trauma complicates and worsens the prognosis in retinal detachment repair Seven patients in whom poor visualization of the fundus seemed to preclude success by routine procedures underwent combined vitrectomy scleral buckling release of subretinal fluid, and intravitreal gas injection The retina was successfully reattached in five of the seven patients although postoperative visual acuity was limited possible on account of the duration of retinal detachment prior to repair or because of the degree of macular involvement

Key words retina – detachment scleral buckling – vitreous – vitrectomy – gas injection

Since Gonin (1920) described the management of rhegmatogenous retinal detachment various techniques and modifications have been described to reattach the sensory retina (Arruga 1958 Gibis 1964 Custodis 1951 Gonin 1934 Lincoff Baras and McLean 1965 Meyer Schwickerath 1954 Norton and Machemer 1971 Rosengren 1910 Schepens Okamura and Brockhurst 1957)

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Table

Results of Traumatic Retinal Detachment Treatment

Case Age (yr) Race Sex	Diagnosis or Condition Requiring Retinal Detachment Procedure	Surgical Procedure	Complications and Postoperative Course	Visual Acuity		Factors Limiting Visual Acuity	Follow up (mths)
				Pre operative	Best Post operative		
1 60 W M	18 mths after cataract extraction with vitreous loss pars plana vitreous aspiration and inadvertent retinal perforation total retinal detachment massive preretinal retraction	Vitrectomy scleral buckling drainage of subretinal fluid and air injection	Progressive severe massive preretinal organization and phthisis	HM	LP	Retina remained detached	12
2 [illegible]2 W M	4 mths after corneoscleral laceration with traumatic retinal detachment with massive retraction and vitreous hemorrhage	Vitrectomy and lensectomy scleral buckling drainage of subretinal fluid C_3F_8 injection cryocoagulation of tears	Retina reattached	HM	90/400	Residual preretinal membrane	7
3 31 W M	6 wks after iridocyclectomy with massive vitreous retraction	Vitrectomy scleral buckling drainage of subretinal fluid SF_6 injection cryocoagulation of tears	Retina reattached	HM	20/400	Preexisting macular star fold	6

4	61	W	M	6 wks after phacoemulsification with vitreous loss giant retinal tear vitreous hemorrhage and massive preretinal retraction developed 1? days postoperatively	Vitrectomy and membranectomy scleral buckling SF_6 injection transscleral diathermy	Progressive severe massive preretinal organization	HM	LL	Retina remained detached	6
5	10	B	M	11 mths after penetrating trauma with retinal detachment and vitreous membranes and bands	Vitrectomy scleral buckling C_4F_8 injection cryocoagulation of tears	Retina reattached	LP	CF 2 ft	Optic atrophy?	5
6	?0	W	M	15 yrs after penetrating trauma 6 yrs after traumatic cataract extraction with retinal dialysis secondary membrane vitreous membranes and hemorrhage and a fixed retinal fold	Vitrectomy and membranectomy scleral buckling drainage of subretinal fluid C_4F_8 injection cryocoagulation of tears	Retina reattached	HM	0/400	Amblyopia	4
7	14	PR	M	? mths after penetrating trauma with retinal dialysis and vitreous bands causing retinal traction	Vitrectomy scleral buckling drainage of subretinal fluid SF_6 injection	Retina reattached	CF 3 ft	0/?0	Macular hemorrhage	3

Although the success rate to cure retinal detachment reaches 95 % for primary detachment (McPherson and Moura 1974) it is not as great in reoperations and when preretinal organization (Criswick and Brockhurst 1969) ocular trauma (Freeman Cox and Schepens 1974 Malbran Dodos and Hulshus 1972) or giant tears (Freeman Schepens and Couvillon 1970) are present We have performed a combined procedure – vitrectomy scleral buckling release of subretinal fluid and intravitreal gas injection – in patients in whom poor visualization of the fundus after ocular trauma seemed to preclude success by routine procedures

Technique

With the patient under general anesthesia a large canthotomy and a 360 degree peritomy are done and bridle sutures (4–0 black silk) are passed underneath the four isolated rectus muscles After localization of the visible breaks a nondetached area either superior temporal or inferior temporal is chosen for pars plana sclerotomy A cataractous lens or any secondary membranes are removed prior to vitrectomy The vitreous opacities vitreous hemorrhage bands and membranes are cut and removed with the vitrophage (Peyman and Dodich 1971 Peyman and Huamonte 1975) After removal of the vitrophage the pars plana sclerotomy is closed and the visible breaks or tears are again localized An exoplant or implant is temporarily secured to the sclera with mattress sutures (4–0 Dacron) An encircling band is also used routinely

After release of subretinal fluid though a second sclerotomy site the intraocular fluid is exchanged for gas (C_4F_8 40 % and air 60 % or SF_6 40 % and air 60 %) Gas is injected through a 25 gauge needle either through the cornea in an aphakic eye or through the pars plana in the phakic eye The fluid is removed via a needle inserted inside the eye through the pars plana sclerotomy Cryocoagulation is applied to all retinal breaks under direct visualization by indirect ophthalmoscopy Finally the exoplant or implant sutures are tightened to create a moderate buckle The intraocular pressure should not be above 15 mm Hg Postoperatively the patients are positioned according to the location of the retinal breaks so that the gas tamponades the retina against the buckle

Clinical Data

Seven eyes with retinal detachment of traumatic origin had vitreous involvement in the form of vitreous hemorrhage band and membrane formation or preretinal organization (Table) All had penetrating injuries with areas of

fixed folds Following is a typical case of penetrating ocular trauma with retinal detachment secondary membranes and vitreous bands and membranes

Case History

A 20 year old white man (Case 6) had injured his left eye with a pencil at the age of 5 Repair of the corneal laceration subsequently led to traumatic cataract which was removed at the age of 14 He had low vision for one month prior to our examination Ocular examination on Jan 19 1975 revealed visual acuity of 20/20 in the right eye and hand motions at 2 feet in the left eye and left exotropia of 16 degrees A linear leukoma was adherent at the inferior cornea Iris prolapsed through the healed wound in the limbus at 11 o clock A moderately dense secondary membrane with Elschnig pearls was visible in the posterior chamber Indirect ophthalmoscopy revealed total retinal detachment with balloons and folds at the posterior pole and a fixed star fold temporal to the macula A secondary membrane at the anterior segment as well as corneal scarring vitreous membranes and hemorrhage precluded visualization of the peripheral fundus

On Jan 21 a pars plana membranectomy and vitrectomy as well as lysis of iridocorneal adhesions were performed and clear media obtained Pars plana sclerotomy was done at the upper temporal quadrant Indirect ophthalmoscopy revealed a retinal dialysis in the upper nasal quadrant from 10 to 11 30 o clock and a horseshoe tear just posterior to the nasal end of the retinal dialysis Diathermy marks were placed on the sclera and the retinal dialysis and retinal break were cryocoagulated A 276 silicone grooved plate was placed from 9 to 12 30 o clock and an encircling band was passed The procedure was completed as described above At the end of the procedure the star fold temporal to the macula remained unchanged Postoperatively the patient developed transient elevation of intraocular pressure which was controlled with acetazolamide Three months after operation the retina remained attached the star fold temporal to the macula had flattened and vision was 20/400

Discussion

Since treatment of retinal detachment should be tailored to the extent of detachment and tears routine procedures such as scleral buckling and cryocoagulation with or without drainage of subretinal fluid, often do not suffice in traumatic retinal detachment Retinal detachment is often accompanied by vitreous hemorrhage, bands membranes and massive preretinal retraction

In addition lens opacities or secondary membranes may obscure the visualization of the fundus Four of our patients (Nos 2 4 6 and 7) had traumatic cataract and secondary membranes In the past when anterior segment opacities accompanied a retinal detachment a two step procedure was advocated – first removal of the cataract or excision of the secondary membranes and second the retinal detachment repair at a later date if possible Unfortunately the vitreous involvement as a result of penetrating trauma complicates and worsens the prognosis of a retinal detachment repair Often the retinal breaks and tears are obscured by vitreous opacities and escape detection Removal of cataracts secondary membranes and vitreous opacities by the vitrophage not only facilitates observation but also releases traction and allows reattachment of the retina Vitrectomy combined either with scleral buckling alone or with scleral buckling and gas injection has been described for treatment of retinal detachment (Machemer and Norton 1972) If only a scleral buckle is used after vitrectomy in patients with fixed folds and preretinal organization retinal breaks or tears may remain open If only gas is injected into the vitreous cavity this may not suffice to reattach the retina because of fixed folds or thick retina We think that in desperate cases of traumatic retinal detachment due to penetrating injury this combined procedure – pars plana vitrectomy scleral buckling release of subretinal fluid and intravitreal gas injection – offers a better chance for retinal reattachment

In our preliminary series we reattached five of seven retinal detachments due to penetrating traumas Unfortunately the visual outcome in these cases was not as promising We believe that two factors greatly influence the visual prognosis in these eyes – first the duration of retinal detachment prior to repair and second the degree of macula involved or fixed folds Early vitrectomy and repair of retinal detachment should improve the prognosis

Acknowledgment

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EVOLUTION OF DESCENDING OPTIC ATROPHY

A case report

BY

MATS LUNDSTRÖM and LARS FRISÉN

Fundus changes following severe trauma to the intracranial optic nerve were followed by means of serial fundus photography The eye was completely blind Little change was seen during the first 4 weeks The retinal nerve fibre layer disappeared gradually during weeks 4 to 8 At the same time the retinal vessels turned narrow and vascular pseudo sheathing appeared close to the optic disc Disc pallor was not maximal until the 12th week when the peripapillary retina also had acquired a mottled appearance

Key words optic nerve – optic atrophy – nerve injury – ophthalmoscopy – fundus photography – retinal anatomy

Although frequently seen by every ophthalmologist little objective information is available on the funduscopic appearance of different stages of optic atrophy and there is room for conflicting views on the time course of evolving atrophy We have had the opportunity to document by serial ophthalmoscopy and fundus photography the evolution of descending optic atrophy following severe injury of the intracranial optic nerve Our observations of the time course of changes in the optic disc the peripapillary retinal nerve fibre layer the retinal vessels and the retinal texture are given in the following

Received May 30 1975

Case Report

Intent on suicide a 35 year old police officer shot himself with his 7 65 mm service gun He put the gun into his mouth aimed upwards and fired He was found unconscious bleeding from the mouth and the forehead and was admitted to Sahlgrenska sjukhuset He was immediately brought to the neurosurgery operating theatre given transfusions and was explored surgically through a left frontal approach The bullet was found to have entered the skull in the left anterior fossa 5 mm from the midline medial to the optic canal The left optic nerve appeared intact The right optic nerve was not visualized Some bleeding from the frontal lobe was easily stopped as was the bleeding from the exit wound situated about 9 mm to the left of the superior sagittal sinus Following surgery it was observed that the right pupil did not react at all to direct light stimulation although the consensual reaction was normal Because of obtundation vision testing was not possible until the 12th day The right eye was amaurotic at that time and there was an absolute upper temporal cut in the left visual field. Visual acuity was 1 0 in the left eye These observations indicated injury to the right anterior knee of the optic chiasm presumably caused by a small bone splinter Repeated Polytome tomograms of the optic canal area were consistently normal except for clouding of the ethmoidal cell system Vision did not improve during the 5 months of follow up

Because of pronounced eye lid swelling careful ophthalmoscopy was not possible until day 5 and useful fundus photographs could not be obtained until day 12 These examinations were repeated at least twice a week for the following 9 months and about once weekly for another 2 months The patient was able to resume his occupation 8 weeks following his suicide attempt He had no neurological sequelae besides the visual handicap

We used a Carl Zeiss Fundus Camera with its 2X magnification attachment Black and white negative film (Kodak Tri X Pan) was exposed through a built in interference filter with a maximal transmission at 490 nm and a spectral half width of 95 nm This is a red free filter it serves to enhance the retinal nerve fibre layer (Behrendt & Wilson 1965) The negatives were developed in Kodak Microdol 1 3 and were printed on Kodak Bromide Paper 3 Dodging or other techniques for contrast manipulation were not used Colour diapositives (Kodachrome II) were also obtained

Results

Our major observations are documented in the following fundus photographs (Figs 1–4) obtained on days 12 25 47 85 The major changes are described in the captions to these Figures Additional observations are detailed in the review following the picture pages For lack of space the right eye only (the blind eye) is reproduced here The changes seen in the left eye were similar in nature but restricted to the lower nasal area in agreement with the upper temporal field defect of this eye

The temporal course of various fundus changes is summarized in Fig 5

During the first 2 weeks following injury a slight bilateral disc oedema was



Fig 1

Twelve days following injury there is evidence of a slight disc oedema with hyperemia of the optic disc a thick and blurred peripapillary nerve fibre layer and wide slightly tortuous vessels with a poor axial reflex Note the prominence of the more peripheral parts of the nerve fibre layer which appears somewhat coarsely striated and slightly opaque hiding segments of larger and smaller vessels

seen (Fig 1) This was very likely a corollary of increased intracranial pressure and should therefore properly be labelled papilloedema rather than disc oedema The intracranial pressure was not actually recorded during this interval but the clinical state described above supports this interpretation The papilloedema



Fig 2

On the 25th day the fundus picture is extensively normalized The slight haziness of the disc border is the only visible abnormality at this stage There is no evidence of hyperemia or stasis The nerve fibre layer is now finely striated throughout and the optic cup appears normal Small vessels now have a normal diameter and are partially obscured by overlying nerve fibre bundles (arrows) cf also with Fig 1 where stasis enhances these vessels in spite of the simultaneous increase in nerve fibre blur

disappeared during the third week and observations on days 20 25 and 30 showed no funduscopic abnormality except for decreasing haziness of the disc border (Fig 2) Around day 30 the nerve fibre layer started to change succes

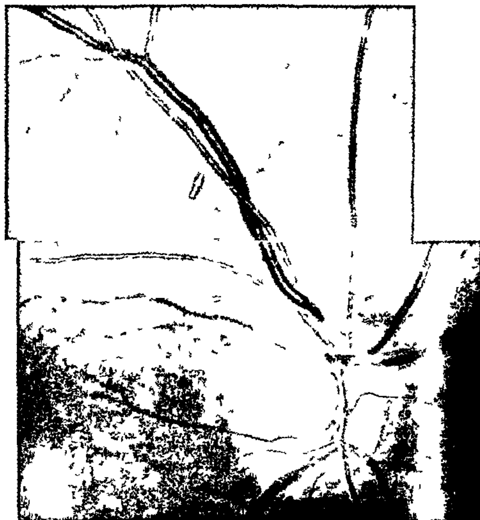


Fig 3

Forty seven days following injury the disc tissue still appears normal in spite of the severe reduction in nerve fibre layer opacity The disc border is now denuded exposing small pigment clumps along its circumference Some fine striations are still visible below the vascular arcade and small vessel cross hatching still exists here (arrow) The walls of major vessels are partially exposed close by the disc (pseudo sheathing)

sively melting down and disappearing nearly completely during the following 17 days This was evidenced by decreased preretinal opacity loss of superficial radial striation exposure of small epi retinal vessels previously hidden in the nerve fibre layer and a slight enhancement of the choroidal pattern (Fig 3)



Fig 4

On the 85th day the disc is distinctly pale, and its border is now abnormally conspicuous all around its circumference because of an apparently complete loss of retinal nerve fibres Striations can nowhere be seen and small vessels are clearly defined throughout their course There is an overall reduction of vascular diameters and still more pronounced pseudo sheathing of major vessels The fundus has aquired a finely granular mottled appearance

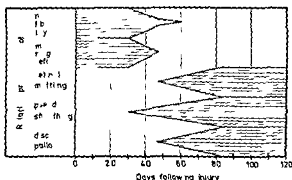


Fig 9

Scheme of time course of fundus changes in evolving optic atrophy

There was a simultaneous slow break down of the macular ring reflex (Figs 6A and B) The last vestige of the arcuate bundles disappeared around day 60 Long before this stage the retinal vessels had assumed a smaller diameter and a straighter course Conspicuous pallor of the optic disc was not seen until day 85 Interestingly enough peripapillary changes were still occurring after day 60 namely the appearance of a mottled fundus granularity No further changes were seen from day 85

The cup/disc ratio increased from 0 6 to 0 7 between the 3rd and the 12th weeks

The changes seen in the left eye although less extensive were time locked to those occurring in the right eye

Discussion

Descending optic atrophy was late in appearance in the eye ground as no changes indicative of atrophy were seen until day 30 The retinal nerve fibre layer then started to melt down and was invisible 30 days later The greater part of the nerve fibre layer was lost in about 3 weeks More protracted changes were seen in the arcuate bundles it is possible that these late changes reflect a reorganization of the internal limiting membrane rather than late nerve fibre wasting Anderson (1973) studying the changes in the ocular fundus following experimental transection of the optic nerve in squirrel monkeys observed a more rapid wasting of retinal nerve fibres This may represent a species difference

The fact that the changes seen here were simultaneous in the two eyes further

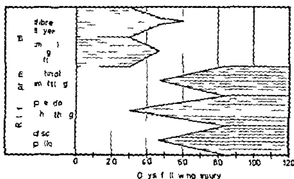


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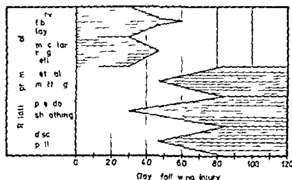


Fig 5

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The cup/disc ratio increased from 0 6 to 0 7 between the 3rd and the 17th weeks

The changes seen in the left eye although less extensive were time locked to those occurring in the right eye

Discussion

Descending optic atrophy was late in appearance in the eye ground as no changes indicative of atrophy were seen until day 30 The retinal nerve fibre layer then started to melt down and was invisible 30 days later The greater part of the nerve fibre layer was lost in about 3 weeks More protracted changes were seen in the arcuate bundles it is possible that these late changes reflect a reorganization of the internal limiting membrane rather than late nerve fibre wasting Anderson (1973) studying the changes in the ocular fundus following experimental transection of the optic nerve in squirrel monkeys observed a more rapid wasting of retinal nerve fibres This may represent a species difference

The fact that the changes seen here were *simultaneous* in the two eyes further

attests to the phenomenon that nerve fibre degeneration does not occur gradually along the nerve but occurs along all of the nerve at the same time (Anderson 1973) The left eye would otherwise have showed more protracted changes as the degenerating fibres of this eye pass the longer route through the optic chiasm

The dynamic fundus changes documented here lend support to the interpretations advocated by Hoyt et al (1973) of static abnormalities in the retinal nerve fibre layer in glaucoma and other disorders of the optic nerve Histological confirmation is still scarce however (Frisch et al 1974) The evolution of fundus changes was not terminated by the macroscopic disappearance of the nerve fibre layer Disc pallor was much later in appearance and was not suspected until day 60 Pallor was maximal first around day 85 Disc pallor obviously cannot be ascribed solely to a disappearance of retinal nerve fibres The ultimate cause of pallor is not known but it appears from these observations that additional changes must take place Perhaps these changes consist in a reorganization of the glial matrix or the myelin sheaths a reorganization that changes the light reflecting properties of the optic nerve head

Pallor is a late and often debatable clinical sign of optic atrophy particularly in cases with only partial damage of the optic nerve Attention to the appearance of the peripapillary retina frequently can contribute to the clinical diagnosis (Hoyt et al 1973 Frisen & Hoyt 1974 Lundström 1974)

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CORNEAL THICKNESS AFTER CATARACT EXTRACTION WITH AIR IN THE ANTERIOR CHAMBER

BY

M S NORN

A total of 135 patients had the corneal thickness measured prior to cataract extraction The chamber was re established by air in 86 while the remainder acted as controls On the second postoperative day the corneal thickness had increased by 12 7 % in the air inflated group and by 17 6 % in the control group On the sixth postoperative day the increases in thickness were 7 2 and 11 1 % respectively On follow up control 6 to 12 months later the corneal thickness was as before the operation Epithelial oedema was significantly more frequent in the control group

The conclusion is drawn that an air bubble in the anterior chamber has no unfavourable effect on the corneal endothelium On the contrary it seems to protect the endothelium from postoperative damage

Key words cornea – corneal endothelium corneal thickness – pachymetry – air in chamber – cataract extraction

Some surgeons prefer to fill the anterior chamber with air at the conclusion of an operation (cataract extraction corneal grafting) while others advise against inflation with air

Adherents of the procedure of air inflation in the chamber attach importance to the re establishment of the anterior chamber immediately after the opera

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CORNEAL THICKNESS AFTER CATARACT EXTRACTION WITH AIR IN THE ANTERIOR CHAMBER

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M S NORN

A total of 135 patients had the corneal thickness measured prior to cataract extraction. The chamber was re-established by air in 86, while the remainder acted as controls. On the second postoperative day the corneal thickness had increased by 12.[illegible] % in the air inflated group and by 17.6 % in the control group. On the sixth postoperative day the increases in thickness were [illegible]2 and 11.1 % respectively. On follow-up control 6 to 12 months later the corneal thickness was as before the operation. Epithelial oedema was significantly more frequent in the control group.

The conclusion is drawn that an air bubble in the anterior chamber has no unfavourable effect on the corneal endothelium. On the contrary it seems to protect the endothelium from postoperative damage.

Key words: cornea – corneal endothelium – corneal thickness – pachymetry – air in chamber – cataract extraction

Some surgeons prefer to fill the anterior chamber with air at the conclusion of an operation (cataract extraction, corneal grafting) while others advise against inflation with air.

Adherents of the procedure of air inflation in the chamber attach importance to the re-establishment of the anterior chamber immediately after the opera-

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tion Those opposed to this procedure fear pupillary block with consequent secondary glaucoma caused by the air bubble

The air bubble may have an unfavourable effect on the corneal endothelium by impairing its vitality or its permeability Leibowitz et al (1974) in rabbit experiments showed that scattered endothelial cells might be damaged when air was introduced into the chamber Conversely von Bahr (1956) in short rabbit experiments found no alteration of the corneal thickness when aqueous humour was replaced by air

On the other hand the air bubble may protect the endothelium from post operative damage of the high protein secondary aqueous humour of fibrin blood and a possibly prolapsing vitreous body The air bubble further prevents any penetration of fluid into the region it covers

The corneal thickness measured with a Haag Streit pachymeter is a sensitive indicator of alterations in endothelial permeability e g after corneal grafting (Ehlers 1974)

It therefore seems natural in an attempt to find whether the air bubble has a favourable or harmful effect to perform pachymetry in relation to cataract extraction both with and without air inflation into the anterior chamber

Method and Material

Cataract extraction was performed with a limbus based conjunctival flap and five corneo scleral knotted silk sutures 8-0 as described previously (Norn 1973)

At the conclusion of the operation atmospheric air was inflated into the anterior chamber with a syringe with a blunt canula The chamber was thereby re established We aimed at inflating just so much air that the air bubble had a diameter of not less than 6-8 mm We endeavoured to avoid a rise in pressure or air in the posterior chamber

All the author s cataract operations from October 1972 to August 1974 were included consecutively in the material under review Air was inflated at the end of operation every other month

A total of 86 operations were performed with air inflation and 49 without

The corneal thickness was measured with a Haag Streit pachymeter mounted on a Haag Streit slit lamp no 900 as described previously (Norn 1973) The measurements were undertaken the day before and on the second and the sixth day after operation Finally the patients were summoned to a follow up with measurement 6 to 12 months after the operation

Ten per cent of those who had air inflated and 10 % of the controls failed to appear for follow up examination (14 patients of whom five had died and three lived abroad while the remaining six were too weak to come to the hospital unit for a control)

Results

A few days after operation the cornea was seen to have increased considerably in thickness The increase was greatest in the control group The percentage increase in proportion to the preoperative value is shown in Table I The highest value was measured on the second postoperative day and a somewhat lower value on the sixth day On measurement 6 to 12 months after the operation the thickness did not differ significantly from the preoperative measurement

A significant difference in corneal thickness was noticed between the patients with air inflated into the chamber and the control group both on the second postoperative day ($P < 0\ 01$) and on the sixth postoperative day ($P < 0\ 05$ Students t test)

Likewise oedema of the corneal epithelium was most frequently present in the control group 51 % on the second postoperative day as opposed to 26 % in the air inflated group ($P < 0\ 01$) 22 % on the sixth day as opposed to 8 % ($P < 0\ 05$) At the follow up epithelial oedema was found in only one case from each group

Within the air inflated group 33 (38 %) still had an air bubble in the chamber on the second postoperative day In four of these the bubble was a small one situated behind the iris plane

On the sixth day air was present in one case only There was no instance of pupillary block.

Table I

The mean percentage increase of the corneal thickness after cataract extraction ± SEM in 86 patients operated on with air inflated into the anterior chamber and 49 patients without air inflation.

	Air	Controls
2nd postoperative day	12 , ± 1 1	17 6 ± 1 7
6th postoperative day	7 2 ± 0 9	11 1 ± 1 4
After 6–12 months	/0 2 ± 0 8	´0 1 ± 1 1

Discussion

The two series are comparable all the operations having been performed by the author according to the same technique and within the same period

The two series showed no difference with regard to incidence of complica tions (few cases of haemorrhage in chamber vitreous cornea contact loss of vitreous and flat chambers in both groups)

The investigation showed that the postoperative increase of the corneal thickness is lessened if air is inflated in to the anterior chamber The effect is seen even after absorption of the air bubble

The air bubble seems to have no unfavourable influence on the vitality of the endothelium On the contrary it seems to protect the endothelium from the harmful effects of the operation

The favourable effect of the air bubble can possibly be intensified by using a more slowly absorbable non toxic gas (perhaps SF_6) This would however probably add to the risk of air bubble induced pupillary block

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DISTRIBUTION OF ASCORBIC ACID IN THE CILIARY BODY OF ALBINO RABBIT, GUINEA PIG AND RAT

BY

AMUND RINGVOLD

The distribution of ascorbic acid in the ciliary body has been demonstrated by means of a silver nitrate technique. It appeared that the basal cells in the pars plana region and in the valleys between the ciliary processes contain great amounts of ascorbic acid in contrast to the basal cells over the processes proper which showed only traces. Rabbit and guinea pig revealed mainly the same findings and quantitative differences occurred before and after intracardial injection of ascorbic acid. On the other hand ascorbic acid was absent in the ciliary body of untreated rats, whereas only traces were found after intracardial injection of this substance. These species differences are in keeping with previous reports showing high concentration of ascorbic acid in the aqueous humour from rabbit/guinea pig and no ascorbic acid at all in aqueous humour from rats.

Key words: ciliary body – ascorbic acid – light microscopy – rabbit – guinea pig – rat

The high ascorbic acid (AA) content in the aqueous humour compared with that in the serum of many species has been extensively studied, and it has been suggested that this condition is brought about by a concentrating mechanism in the ciliary body. Since subnormal AA values were found in the aqueous humour from aphakic eyes by some authors (Muller & Buschke 1934, Langham 1950, Boyd 1955, Heath et al. 1961) the hypothesis was formulated (Friedenwald et al. 1943) that dehydroascorbic acid (DHA) entering the posterior

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chamber through the ciliary epithelium is transformed by the lens to AA which is a less diffusible form This view was supported by other authors (Langham 1950 Heath et al 1961) and by early histochemical studies indicating lack of AA within the epithelial cells of the ciliary body (Friedenwald et al 1943 Schmid & Burki 1943) In contrast to this Kinsey (1950) was unable to show conversion of DHA to AA by the lens *in vivo* and *in vitro* and accordingly he argued that AA is secreted by the ciliary epithelium into the posterior chamber in reduced form According to Oda (1968) histochemical findings in rabbit ciliary epithelium support this interpretation However the latter report refers to the ciliary processes only without stating specifically in which layer of the epithelium AA was found Since a pilot study with Chinoy s modification (*Chinoy 1969*) *of the silver method for demonstration of AA revealed marked* regional differences in the ciliary body of an albino guinea pig it was decided to extend the investigation and compare results from species known to have *high concentration of AA with those from species that have no AA in the* aqueous humour

Material and Methods

Both eyes were used in all animals

Rabbits Five adult albino animals weighing 2 5–3 kg were used They were kept on pelleted diet number 176 (Møllesentralen a/s Oslo) and water *ad libitum* Anaesthesia was performed with about 100 mg pentobarbitone sodium injected intravenously In two animals the eyes were enucleated without any further experimental procedures In three rabbits one eye was removed then

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Fig 1

(Rabbit before AA injection Araldite embedding) a) Black silver deposits are seen in basal epithelial cells of the pars plana region Silver precipitates are also present on the apical cell surface towards the posterior chamber (arrow) Note artificial discontinuity of the apical cell layer in the pars plana region C cornea PC posterior chamber × 50 b) Detail from the boxed area in Fig 1a showing black silver grains in basal epithelial cells Meridional section × 700

Fig 2

(Rabbit after AA injection Araldite embedding) a) Distinct silver deposits in basal epithelial cells both in pars plana and in the valleys between the ciliary processes (arrows) *Note silver precipitates are lacking in basal cells covering the most prominent* part of the processes × 50 b) Detail from the boxed area in Fig 2a Silver grains are accumulated in the cell cytoplasm towards the ciliary stroma *Meridional section* × 700



Fig 1



Fig 2

300 mg AA in 3 ml 0.15 M saline solution was injected intracardially and 10–15 min later the other eye was removed

Guinea pigs Ten albino animals weighing 250–300 g were studied They were fed as above and in addition their water contained about 1 mg AA/ml Intraperitoneal injection of 12–15 mg pentobarbitone sodium was used as anaesthesia In six animals both eyes were removed immediately The remaining four animals were enucleated on one side and the other eye was enucleated 10–15 min after intracardial injection of 20 mg AA in 1 ml 0.15 M saline solution The intracardial injection was made directly after thoracotomy with artificial respiration through a tracheostomy

Rats Four albino rats weighing 180–200 g were studied They were fed pelleted diet number 153 (Møllesentralen a/s Oslo) and water *ad libitum* Initial ether anaesthesia was followed with about 10 mg pentobarbitone sodium intraperitoneally after which one eye was removed Ten mg AA in 1 ml 0.15 M saline was then injected intracardially (application as described for guinea pigs) and the second eye was removed 10–15 min later

All eyes were treated in the same way The anterior segment was cut off posterior to ora serrata the lens was carefully extracted from behind and the specimens were put into precooled acid silver nitrate solution in dark bottles according to Chinoy (1969) After 24 hours the tissues were rinsed thoroughly in the prescribed solution dehydrated in alcohol and embedded in paraffin or Araldite Paraffin (7 μm thick) and Araldite (2 μm thick) sections were stained with cresyl violet and toluidine blue respectively and mounted under cover glasses

Results

In this paper the terms basal and apical cells (layers) will be used for cells (layers) facing the ciliary stroma and the posterior chamber respectively

Rabbits The amount of silver precipitate differed considerably from one section to the other within the same eye and silver grains were present both within the epithelial cells and in the connective tissue of the ciliary body In the epithelium large amounts of silver grains were frequently found within the basal cells in the pars plana and in the valleys between the ciliary or iris processes (Figs 1 a 2 a and 3) Usually the silver deposits were concentrated in the basal part of the cells (Figs 1 b 2 b) sometimes apparently localized

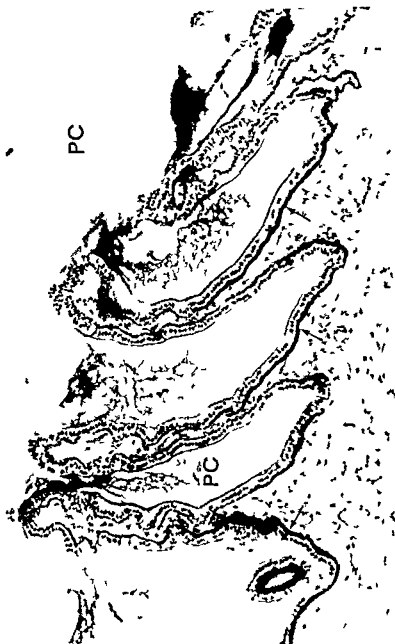


Fig 3
(Rabbit after AA injection Paraffin embedding) Distinct silver deposits are present in basal epithelial cells (arrows) of the valleys between ciliary processes and in the posterior chamber (PC) Note no silver precipitates in basal epithelial cells over the most prominent part of the processes Circular section just anterior to the pars plana region ×150

predominantly in the vicinity of the cell membrane facing the ciliary stroma These phenomena however may be streaming artefacts brought about by the preparation procedure *In contrast to this the basal epithelial cells covering* the processes proper contained only a few silver grains which were randomly scattered throughout the cytoplasm The apical cell layer revealed only scattered grains in all regions and it should be stressed that heavy silver deposits were never observed in any part of this layer Distinct deposits were often *present however on the posterior chamber cell surface* (Fig 1 a) *Dark brown* deposits were particularly abundant along the inner surface of pars plana probably due to remnants of vitreous body material (cp Fig 5 a) The apical cell layer often showed discontinuities in the pars plana region (Fig 1 a b) whereas such cell damage was seldom observed on or between the processes In the *connective tissue just beneath the basal cells of pars plana a thin band of silver* deposits was frequently seen In addition moderate diffuse silver deposits occurred in different parts of the ciliary stroma seemingly without any preferred location (Figs 1 a 2 a)

After intracardial injection of AA the distribution of silver deposits appeared *similar to the description above* but *in most sections the precipitates were more* abundant (Fig 2 a) The basal cells over the most prominent part of the ciliary processes and the whole apical cell layer still showed only few scattered silver grains (Figs 2 a 3) Silver grains were also present in vessel lumina in moderate amounts

Electron microscopic investigation indicated that the silver grains are localized to some small vesicular organelles of the ciliary epithelium but due to poor fixation the tissue was unfit for further evaluation with this technique

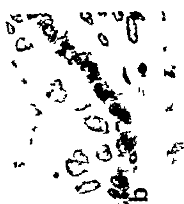
Guinea pigs Sections from this species revealed mainly the same findings *before and after intracardial injection of AA as described for rabbits* (Figs 4 a b and 5 a b)

Fig 4

(Guinea pig before AA injection Araldite embedding) a) Silver deposits localized in the basal epithelial cells of pars plana C cornea PC posterior chamber × 50 b) Detail from boxed area in Fig 4 a Meridional section × 100

Fig 5

(Guinea pig after AA injection Araldite embedding) a) Marked silver deposits in basal epithelial cells of pars plana Note heavy deposits at arrow probably being caused by vitreous body remnants I iris C cornea × 50 b) Detail from the boxed area in Fig 5 a Meridional section × 100





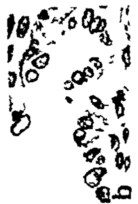


Fig 4 Fig 5 Fig 6

Fig 6

(Rat after AA injection Araldite embedding) a) No distinct silver deposits in any ciliary body region even after AA injection PC posterior chamber I iris C cornea × 50 b) Detail from Fig 6 a (region indicated by arrow) Meridional section × 700

Rats Untreated animals showed no silver precipitates in the ciliary body After intracardial injection of *AA* a few scattered grains were found both in vessel lumina connective tissue and epithelial cells Substantial silver deposits in *epithelial cells similar to the findings in rabbit and guinea pig were not* observed in any part of the rat ciliary body even after AA injection (Fig 6 a b)

Discussion

The silver nitrate technique introduced by Giroud & Le Blond (1936) for histochemical demonstration of AA has been modified by several authors All modifications show reducing substance and unfortunately AA is only one of several tissue components that are able to precipitate metallic silver in a silver nitrate solution The specificity of the method is considerably improved how ever when the reaction is performed in the dark at low pH and low temper ature Under such conditions silver nitrate is still reduced by AA but not by reducing sugars glutathione and amino acids (Chinoy 1969) These obser vations along with the present demonstration that rats lacking AA in the *aqueous humour (Muller & Buschke 1934) revealed no reducing* substance in the ciliary body prior to AA injection indicate that the method applied gives a fairly reliable identification of AA within these tissues Accordingly the present study demonstrates that in both rabbit and guinea pig the basal cells in pars plana and the valleys between the ciliary processes contain much more AA than the basal cells covering the processes proper It is generally accepted that these species contain high concentration of AA both in the ciliary body *and in the aqueous humour and evidence has been brought (Friedenwald et al* 1943) that AA in some way or other influences the aqueous humour secretion The functional significance if any of the regional differences in AA content is at present unknown It is however unlikely that this accumulation of AA refers to synthesis within these particular cells since the AA concentration in aqueous humour decreases rapidly towards zero values in guinea pigs fed for 3 weeks with scorbutic diet (Hughes et al 1971)

It is essential to stress that the silver nitrate method shows AA in reduced form only and consequently the pale tissue regions may well contain DHA Thus studies based on this method cannot settle the question as to whether the substance concerned is secreted into the intraocular fluid as AA or DHA

Previous reports (Muller & Buschke 1934 Langham 1950 Boyd 1955 Heath et al 1961) showing decreased AA level in the aqueous humour some weeks after lens extraction were taken to indicate that lens tissue is necessary to

ensure a high AA concentration Even though the lens was carefully removed from the anterior segment in the present experiments the ciliary body frequently revealed tissue damage particularly in the pars plana epithelium Similar tissue damage has to be expected after a conventional lens extraction in young animals too and the difference in AA level of normal and aphakic eyes could be due to permanent postoperative changes in the ciliary body

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BILATERAL KERATOPATHY AND TYROSINOSIS

BY

HANS OTTO SANDBERG

A case of tyrosinosis due to lack of soluble tyrosine aminotransferase is described The first clinical sign of this disorder may be bilateral kerato pathy Treatment is diet with restriction of phenylalanine and tyrosine The disorder is rare and must be differentiated from other conditions of tyrosinosis

Key words cornea – inborn error of metabolism – soluble tyrosine amino transferase – tyrosine – tyrosinosis

Tyrosinosis due to lack of soluble tyrosine aminotransferase (STAT) is a rare inborn error of metabolism As bilateral keratopathy is one of its most consistent clinical features it deserves the attention of ophthalmologists Five cases have been described so far (Wadman et al 1968 Holston et al 1971 Burns 1972 Goldsmith et al 1973 Zaleski Hill & Murray 1973) In none of these cases has the diagnosis been made as early as in the present case Early diagnosis may be of importance for the growth and development of the patient

Case Report

The patient a girl was born at term after an uneventful pregnancy and delivery She was the first child of parents without known consanguinity Mother and child were dis charged from the maternity ward on the 5th day Ten days later the parents noticed a

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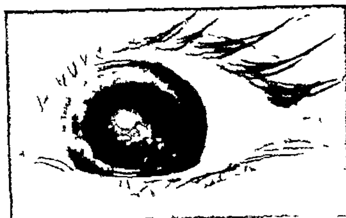


Fig 1

Left eye at the age of 14 weeks The corneal epithelium shows peripheral dendrites while the central infiltrate is less pronounced than earlier The right eye showed the same changes At this time both corneae had been cauterized with iodine 5 times Diet was instituted 1 week later

central opacity in both corneae She was admitted to the eye department 20 days old Her conjunctivae were pale and there was only weak pericorneal injection A central superficial infiltrate with ramifications was found in both corneae The infiltrates were oval 2×4 mm with the long axis horizontal From this infiltrate short dendrites extended in all directions Corresponding to the central infiltrate the epithelium was loosened and crumpled Otherwise nothing pathological was found at clinical examination She was first treated locally with idoxuridine and later with 0 5 % silver nitrate without any effect During a period of 7 weeks both corneae were cauterized with iodine 5 times The infiltrates became smaller but did not disappear (Fig 1) At the age of 15 weeks a diet with reduced phenylalanine and tyrosine was instituted One week afterwards the epithelial lesions in both corneae were healed

When last seen 4 months after diet was instituted a slight nebula was found in her right cornea while no abnormality was found in the left So far her physical and mental development seem to be normal

Laboratory findings

At the time of discharge from the maternity ward a blood sample was examined as part of screening for metabolic disorders Serum tyrosine was markedly increased and Vitamin C was given in addition to the diet This had no effect on the serum tyrosine level which was consistently increased until diet was instituted at the age of 15 weeks After 14 days on diet the serum tyrosine fell from 51 mg % to 10 mg %

Before the diet was started analysis of the urine showed normal para hydroxy phenylpyruvic acid (PHPPA) and increased amounts of para hydroxyphenyl

lactic acid and para hydroxyphenylacetic acid After diet these metabolites became normal At the time of admission to the eye department serum anti herpes simplex virus titers from the patient and her mother were estimated This was repeated after 10 days The mother had stationary titers (40 and 40) and the patient falling titers (20 and 5)

Discussion

Tyrosinosis was first described in a 49 year old male patient who had been diagnosed as a case of myasthenia gravis (Medes 1932) This patient does not clinically resemble any of the later described cases of tyrosinosis Biochemically however there is some resemblance to the cases of tyrosinosis characterized by hepato renal failure in children In the acute form the patients usually die during their first or second year Some patients have a more chronic course and may live up to 20 to 30 years These patients have reduced activity of PHPPA oxidase probably secondary to hepatic cirrhosis (Woolf 1966) The condition is inherited Diet low in phenylalanine and tyrosine is beneficial (Halvorsen & Gjessing 1966)

Another form of tyrosinosis affects premature children In these cases increased tyrosine and tyrosine metabolites are found in the urine during the first 2 to 6 weeks in some even longer at which time their urine spontaneously and suddenly becomes normal This transient condition is caused by immaturity of the PHPPA oxidase as a result of the prematurity (Woolf 1965) Vitamin C can increase the activity of the enzyme and should be given in large doses Neither the tyrosinosis with hepato renal failure nor the tyrosinosis of prematurity is associated with eye symptoms

Five cases of tyrosinosis have been described who have quite another clinical picture The present case is the sixth These patients lack soluble tyrosine amino transferase The defect is thus at an earlier stage in the tyrosine metabolism than in the other conditions where there is a lack of PHPPA oxidase These cases have no hepato renal affection Although an enzyme determination has been performed in only one of the described cases (Fellman et al 1969 Burns 1972) the clinical picture and laboratory findings make this diagnosis well established

Two of the previously described cases had a history of consanguinity in the parents we have not been able to discover this in our patient Multiple congenital anomalies in addition to the metabolic defect have been described in one case (Burns 1972) Such anomalies have not been found in the other four cases or in our own The five cases previously reported have all been severely mentally retarded Three of the patients had skin manifestations variously described as

painful hyperkeratotic lesions of the palms and soles painful palmar and plantar hyperkeratosis and bullous lesions of the palms and soles All skin manifestations disappeared during the first week after a diet with reduced phenylalanine and tyrosine content Our patient has so far shown normal physical and mental development (Halvorsen 1975) and she has not had any skin lesion This may possibly be explained by the early diagnosis The previous cases have been from 18 months to 18 years old when the metabolic defect was diagnosed while our patient was 15 weeks old

Four of the previously described patients have had corneal changes Two presented a clinical picture resembling our case pale conjunctivae central superficial ulcers in both corneae with branch like infiltrates extending radially (Burns 1972 Zaleski Hill & Murray 1973) In both cases the lesions developed during the first 2 weeks after birth Another had a history of severe keratitis that persisted until the age of 9 months (Holston et al 1971) The fourth had at the age of 8 years thick and red conjuntivae with miniature dendrites which were fluorescein negative in both corneae (Goldsmith et al 1973) One patient who had no history of keratitis developed bilateral cataracts at the age of 17 (Wadman et al 1968)

Treatment with diet low in tyrosine and phenylalanine led to remission of the eye lesions in the cases described by Burns (1972) and Goldsmith et al (1973) Corneal cauterization may have had a beneficial effect in one case (Zaleski Hill & Murray 1973) while another case seems to have had a spontaneous remission at the age of 9 months (Holston et al 1971) It is felt that our case responded to the dietary treatment The corneal lesions persisted after 12 weeks of treatment with anti viral and anti bacterial eyedrops and repeated cauterizations while they disappeared during the first week of diet low in tyrosine and phenylalanine

As has been stressed by Burns (1972) and Zaleski et al (1973) ophthalmologists should be aware that bilateral keratitis in a child of a few weeks of age may be caused by tyrosinosis The diagnosis should then be made by amino acid analysis of serum and urine This may be of great importance should early diagnosis and treatment be found to have a beneficial influence on the mental development of the patient

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BACTERIAL FLORA IN RELATION TO CATARACT EXTRACTION

III Postoperative Flora

BY

J A FAHMY S MØLLER and M WEIS BENTZON**

The conjunctival flora of 499 patients operated for cataract was studied on the 4th and 7th postoperative days and compared with the flora examined previously on admission to the hospital and at the time of surgery (Fahmy et al 1975b c) Antibiotics had been administered approximately 18 hours before operation at the conclusion of surgery and then on the 4th postoperative day after the cultures had been taken
After a significant fall in incidence including all kinds of bacteria at the time of surgery (Fahmy et al 1975c) *Staphylococcus albus* showed an increase in incidence on the 4th postoperative day to the level found on admission Corynebacteria and gram negative bacilli likewise showed increasing incidence but not to the level of admission The incidence of *Staphylococcus aureus* and streptococci remained unchanged
On the 7th postoperative day *S aureus* showed a fall in incidence while other bacteria had largely the same incidence as that of the 4th postoperative day
The origin of *S aureus* isolated postoperatively from the conjunctiva was studied and showed that the great majority of strains were similar to those found on the patient s own conjunctiva preoperatively In a few instances *S aureus* could be traced to the patient s own nose or to the noses of some of the nurses

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The air of the wards eye drops or ophthalmic ointments used in the treatment of the patients apparently did not play any role as a source of S aureus infection
Cultures obtained on the 4th postoperative day showed only a minor relationship to the flora of the wound site observed at the conclusion of surgery

Key words conjunctiva – bacteria – flora – cataract extraction – post operative

Previously the bacterial conjunctival flora of 499 patients was studied on two different occasions on admission to the hospital (Fahmy et al 1975b) and at the time of surgery (Fahmy et al 1975c)

The present paper deals with the postoperative flora of the above mentioned patients The conjunctiva was examined on the 4th and 7th postsurgical days and the results were compared with those obtained on admission and during surgery Particular attention has been given to the occurrence of *S aureus* on the conjunctiva postoperatively All strains were bacteriophage typed and compared with those isolated previously Furthermore an attempt has been made to trace the origin of new strains of *S aureus* isolated postoperatively by examining some potential sources of infection such as nasal flora of nursing staff air of wards as well as eye drops and ophthalmic ointments used in the treatment of the patients (Lehrfeld & Donnelly 1948 Pulvertaft 1949 Raub 1953 Smith 1954 Goettsch 1956 Ridley 1958 Allen 1959 1972 Locatcher Khorazo & Gutierrez 1960 1972 Crompton 1961 Hallermann 1963 Allen & Mangiaracine 1964 Cassady 1967 Editorial 1969 Thomsen et al 1970 Jepsen 1972 1974 Ertel et al 1973)

Material and Methods

The material is described in detail elsewhere (Fahmy et al 1975b) and comprises 499 patients operated for senile (or presenile) uncomplicated cataract at the Eye Department of Kommunehospitalet Copenhagen during the period 17 8 1972–16 8 1973 The routine used for taking bacteriological cultures from the patients was as follows Shortly after *admission* (Fahmy et al 1975b) samples were obtained from the conjunctiva (sample A) nose (sample B) and skin of face (sample C) In the *operating theatre* (Fahmy et al 1975c) cultures were taken from the conjunctiva immediately before surgery (sample E) and from the wound site at the termination of operation (sample I)

In the present study cultures from the conjunctiva were usually taken *on the fourth* (sample J) and *seventh* (sample M) *postoperative days* excepting cases operated on Wednesdays where sample J was taken on the 5th postoperative day In cases where

the patients were discharged from the hospital on the 6th postoperative day sample M was obtained that day The cultures were taken from the lower fornical and tarsal conjunctiva with a dry cotton wool swab immediately after the instillation of 0 2 % oxybuprocaine anaesthetic eye drops (benoxinate hydrochloride = Novesin®) as it was found (Fahmy et al 1975a) that the instillation of anaesthetic eye drops immediately before obtaining cultures significantly increased the isolation rate of bacteria Beside the above mentioned samples from the conjunctiva cultures from the nose (sample K) and air of wards (sample L) were taken the same day as sample J Cultures from the nose were recovered by means of wet cotton wool swabs and those from the air (one sample per patient) were obtained by exposing agar plates (13 5 cm) for 1 hour to the air of the wards in which the patients were admitted The plates were placed beside the patients beds Beside these standard samples (J K L M) weekly cultures were taken (when possible) from the noses of the nursing staff as well as from the eye drops and ophthalmic ointments used in the treatment of the patients

The ward structure The Eye Department occupies the ground floor of the left wing of the main building complex of Kommunehospitalet which was built in 1863

The ward is situated between the policlinic and operating room and comprises three 10 bed units one 4 bed unit and two one bed units These single bed units are usually used for the isolation of infected cases

Postoperative care The dressing is changed on the first postoperative day usually by the same surgeon who operated the day before Mydriatics usually 1 % atropine and 10 % metaoxedrine (phenylephrine) are administered during this procedure One per cent scopolamine (hyoscine hydrobromide) eye drops 3 times daily are then prescribed for the rest of the stay On the 4th postoperative day the patients are examined by slit lamp at the policlinic and a mixture of prednisolone framycetin and polymyxin B eye drops (Neocortol®) is usually prescribed (3–4 times daily) The patch is removed the same day

Bacteriological methods The samples were inoculated within 1–2 hours on 5 % blood agar as well as in serum bouillon. The cultures were incubated at 37°C for 48 hours The identification of bacteria was carried out according to the methods described by Cowan & Steel (1965) Bacteriophage typing of *S aureus* was performed at Statens Seruminstitut Copenhagen by the methods of Blair & Williams (1961)

Statistical methods The statistical methods were largely the same as described previously (Fahmy et al 1975c) Pairwise comparisons were carried out between the incidence of bacteria in samples A ~ J E ~ J J ~ M and I ~ J using the McNemar test For the three latter comparisons the dependence on sample A or E has been examined by con sidering sets of three samples viz A E J A J M and E I J

An analysis of variance performed on the logarithms of the number of colonies sampled from the wards has been us d for comparison between the wards

Results

Bacterial flora

Table I shows the bacterial flora as observed on five consecutive samples The flora of the 4th and 6th postoperative days (samples J and M) was dominated

Table I

Incidence of microorganisms isolated from 499 patients on admission at the beginning and end of surgery and on the 4th and 7th postoperative days

Incidence in percent					
	Preoperative flora	Peroperative flora		Postoperative flora	
Microorganisms	Conjunctiva on admission	Conjunctiva beginning of surgery	Wound site end of surgery	Conjunctiva 4th post op day	Conjunctiva 7th post op day
	Sample A	Sample E	Sample I	Sample J	Sample M
Staphylococcus albus	95 4	87 6	56 3	92 4	91 6
Corynebacteria	44 0	3 2	2 6	6 6	3 6
Staphylococcus aureus	14 9	7 4	3 3	6 8	3 8
Streptococcus					
haemolyticus	1 8	0 2		0 4	0 4
non haemolyticus	1 2 } 0 4	} 0 8	0 0	0 4 } 1 0	1 0 } 1 4
faecalis	1 0	0 6		0 2	
Diplococcus pneumoniae	4 0				
Gram negative bacilli					
Escherichia coli	0 3	0 2		0 2	0 4
Bacterium anitratum	1 4				
Enterobacter cloacae	0 8				
Proteus morgani	1 6	0 4		1 0	0 2
Proteus vulgaris	0 4			0 2	
Proteus mirabilis	1 8 } 7 8	1 0 } 1 6	0 8 } 0 9	2 0 } 3 4	1 0 } 3 0
Proteus rettgeri	0 9				
Klebsiella oxytoca	0 6				0 4
Klebsiella rhinoscleromatis	0 2				
Not identified bacteria (total)					
Gram positive	2 4 } 2 5	0 0	0 8 } 1 0	0 2 } 0 2	0 8 } 0 8
Gram negative	0 4		0 9		
No growth	0 0	9 0	39 0	2 2	3 2
Not examined	0 0	0 6	0 8	3 6	3 9

Table II

Correlation between the incidence of microorganisms isolated from 499 patients on admission at the beginning of surgery and on the 4th postoperative day

Micro organisms									Not examined	McNemar s test Level of significance E ~ J
	Sample A	–	–	–	–	+	+	+	+		
	Sample E	–	–	+	+	–	–	+	+		
	Sample J	–	+	–	+	–	+	–	+		
S albus		3	7	2	10	3	44	12	399	19	$P < 0\,0001$
Corynebacteria		254	7	4	0	179	24	10	2	19	$P = 0\,016$
S aureus		401	4	2	0	28	10	15	20	19	n.s *
Streptococci		4 5	2	1	0	17	2	2	1	19	n.s *
Gram negative bacilli		438	2	0	1	23	9	[illegible]	[illegible]	19	$P = 0\,0$[illegible]

Sample A Conjunctiva on admission
Sample E Conjunctiva immediately before surgery
Sample J Conjunctiva on 4th postoperative day
* Not significant

by *S albus* and largely similar to that found at the beginning of surgery (sample E)

Tables II and III correlate the incidence of bacteria isolated postoperatively (samples J and M) with those found at the beginning of surgery (sample E) and on admission (sample A)

When the incidences in samples E and J (beginning of surgery and 4th post operative day) were compared (Table II) it was found that 409 patients had *S albus* in E and J 14 in E only and 51 in J only The difference is significant ($P < 0\,0001$) When the incidences of the other bacteria were matched in the same manner corynebacteria and gram negative bacilli occurred more frequently in *J* than E ($P = 0\,016$ and 0 022) while *S aureus* and streptococci showed no change in incidence

When the procedure was repeated comparing J with M (4th and 7th post operative day after antibiotics had been used) only *S aureus* showed a significant variation i e occurred less frequently in M than J ($P = 0\,0052$) (Table III)

The change in incidence from E to J was found to be independent of the

results of A however the incidences of positive findings in J were generally higher *if A was positive irrespective of the findings in E*

When the incidences in samples A (on admission) and J (4th postoperative day) were compared (Table III) as mentioned above *S albus* showed no significant change in incidence while corynebacteria *S aureus* streptococci and gram negative bacilli were found to occur less frequently in J than A ($P = 0\ 0007$ and $P < 0\ 0001$)

For *S albus* which did not show significant difference in incidence between A and J or J and M a comparison between the number of colonies was carried out as follows the patients were divided into three groups according to the number of colonies viz < 5 6–25 and > 25 The first group also included those cases with growth in serum bouillon but not in agar *Of 435* patients showing *S albus* in A and J 189 had *semiquantatively* the same number of colonies 105 had more colonies in A than J versus 141 patients who had more colonies in J than A The difference is significant ($P = 0\ 0216$) The number of colonies of *S albus* was matched in the same manner in J and M and no significant difference was found

Table III

Correlation between the incidence of microorganisms isolated from 499 patients on admission on the 4th and 7th postoperative days

Micro organisms	Sample A Sample J Sample M	– – –	– – +	– + –	– + +	+ – –	+ – +	+ + –	+ + +	Not examined	McNemar s test Level of significance A ~ J	J ~ M
albus		1	4	1	16	4	11	18	417	27	n s*	n s*
Corynebacteria		251	4	7	0	175	11	21	3	27	$P < 0\ 0001$	n s*
aureus		394	3	1	3	33	4	22	7	27	$P < 0\ 0001$	$P = 0\ 00$[illegible]
treptococci		448	2	1	1	14	3	2	1	27	$P = 0\ 0007$	n s*
ram negative acilli		427	3	1	2	23	2	6	8	27	$P < 0\ 0001$	n s*

Sample A Conjunctiva on admission
Sample J Conjunctiva 4th postoperative day
Sample M *Conjunctiva 7th postoperative day*
* Not significant

Table IV

Correlation between the incidence of microorganisms isolated from 499 patients on the 4th postoperative day and at the end of surgery

Microorganisms	Sample J negative			Sample J positive		
	Sample I positive	Total*	%	*Sample I* positive	Total*	%
S albus	9	20	45 0	265	461	57.5
Corynebacteria	11	448	2 5	2	33	6 1
S aureus	7	447	1 6	11	34	3-4
Streptococci	1	476	0 2	0	5	0 0
Gram negative bacilli	1	464	0 2	3	17	17 6

Sample J Conjunctiva on 4th postoperative day
Sample I Wound site at the end of surgery
* *18 patients were not examined*

In order to examine the relationship of the postoperative flora (samples J and M) to that found on admission (sample A) the material was divided into two groups according to the occurrence of bacteria in sample A From Table III it is seen that 450 patients had *S albus* in A while 22 did not Of the former group 446 patients (99 1 %) showed *S albus* in J and/or M versus 21 patients (95 5 %) of the latter group The difference as examined by χ test was not significant However when the incidences of the other bacteria were examined in the same manner corynebacteria *S aureus* streptococci and gram negative bacilli were found to occur more frequently ($P < 0\ 0001$) in J and/or M if they were present in A

Thus the findings on admission were still relevant for the postoperative findings *even if a reduction in* incidence was obtained before the operation (Fahmy et al 197 5c)

The relationship between sample I (wound site at end of surgery) and sample J (4th postoperative day) has been studied The results are given in Table IV It is seen that the incidences of microorganisms in sample I were generally higher when sample J was positive However since the incidences in sample I were below 50 % (except for *S albus*) irrespective of the findings in J sample J was not found useful as an indicator for I *This relationship was studied in* more detail using phage types of *S aureus* (see below)

Relationship to some potential sources of infection

Three sources have been examined nasal flora of the nursing staff air of wards and eye drops and ophthalmic ointments From the epidemiological point of view only the occurrence of *S aureus* was considered mainly because it is suitable for bacteriophage typing

Nasal flora of nursing staff Thirty one persons have been examined 10 were nurses 13 student nurses while 8 beloned to some other groups of the nursing staff Fifteen persons were examined more than 10 times (279 examinations) while 16 were examined 2–6 times (62 examinations) Seven persons (three nurses and four student nurses) proved to be carriers showing *S aureus* in almost all examinations one nurse showed *S aureus* in 17 out of 35 examinations while in the remaining 23 persons *S aureus* was found only once or not at all

Table V

Number of airborne bacteria carrying particles per agar plate (13 5 cm)/hours as found on the wards

Colony count (per agar plate and hour)	Number of specimens from air of wards (sample L)							
	Ward							Total
	a	b	c	d	e	f	*	
None								
≤ 10	1			5	1			[illegible]
11–20	8	6	2					16
21–40	36	16	1	4	1	3		[illegible]
41–80	41	30	37	7	1		1	[illegible]
81–160	46	41	43	14	1		2	147
161–320	33	21	39	4	1		2	100
321–640	9	5	5					19
Not examined	8	13	9	6				36
Total	16	137	152	40	5	3	5	[illegible]

Ward a b c = 10-bed unit
d = 4-bed unit
e f = 1-bed unit

* Origin of the sample not recorded

Table VI

Number and kinds of examined eye drops and ophthalmic ointment

Medicaments	Number of bottles/tubes	Number of examinations			
		1	2	≥ 3	Total
Eye drops					
Scopolamine	88	66	17	5	118
Atropine	38	13	12	13	89
Metaoxydrine	61	26	16	19	121
Oxybuprocaine	50	31	16	3	72
Neocortol®	111	78	25	8	153
Others	47	24	8	15	95
Total	395	238	94	63	648
Ophthalmic ointments					
Terramycin polymyxin B	29	7	7	15	77
Others	7	6		1	10
Total	36	13	7	16	87

The strains of *S. aureus* were bacteriophage typed only when the patients showed postoperatively (samples J or M) a phage type complex not similar to that found in previous samples (A, B, C, E, I) or in cases in which *S. aureus* was not isolated previously but occurred postoperatively. The results are described below.

Air flora of wards. Table V shows the number of airborne bacteria carrying particles per plate and hour in sample L. The mean count per plate was about 129 colonies, which, assuming an average settling velocity of 20 m per hour (Thomsen et al. 1970, Jepsen 1972) and area of plate (143 cm^2), corresponds to 451 bacteria carrying particles per m^3. *S. aureus* was isolated in only eight out of 463 examined agar plates (1.7 %). In all cases *S. aureus* was bacteriophage typed and compared with the conjunctival findings (samples J, M). In no instance could a relationship be found. The same applied to gram negative bacilli (10 cases) and streptococci (2 cases) recovered from the air.

The distribution of the number of colonies sampled from the air of the different wards showed a normal logarithmic distribution. An analysis of variance

showed that the variation between the wards (10 bed units versus four or less bed units) was significant as compared to the variation within the wards of the same size (*F-test* F = 4 16 with 4 and 453 degrees of freedom $P = 0.001$–0.005) On the average 91 colonies were found for 10 bed wards against 57 colonies for wards with four beds or less

Sterility of eye drops and ophthalmic ointments Table VI shows that a total number of 395 bottles and 36 tubes have been examined It is seen (Table VI) that 238 bottles and 13 tubes were examined once 94 bottles and 7 tubes twice while 63 bottles and 16 tubes were examined three or more times This made a total of 735 examinations

Ten bottles all prepared according to the *Pharmacopoeia Nordica* (aseptically prepared with addition of 0 5 % phenylaethyl alcohol as preservative and sterilized) at the hospital pharmacy were found to be contaminated with *S albus* Nine out of the ten bottles contained 1 % scopolamine eye drops (hyoscine hydrobromide) and one 0 4 % oxybuprocaine anaesthetic eye drops (benoxinate hydrochloride) The ten contaminations were observed with intervals of about 1 month (November 1972 – July 1973) However when eight out of the 10 bottles were reexamined immediately after the results of the cultures were known (24–48 hours) only two showed contamination with *S albus*

Epidemiology of S aureus

Table VII shows the distribution of phage type complexes of *S aureus* isolated during the different stages of the study Of 34 strains isolated on the *4th postoperative day* (sample J) 27 had either the same phage type complex (23 cases) or were not typable (4 cases) as those strains recovered on admission (sample A) Two strains both non typable could be traced to the operating theatre i e were found on the conjunctival samples recovered at the time of surgery (samples E and I) Of the remaining five strains which were not found on the conjunctival samples A E I three could be traced to the patient s own nose (sample B) and two to the nose of two different nurses

In order to assess the ability to redetect bacteria found on the wound site at the conclusion of surgery (sample I) in cultures taken on the 4th postoperative day (sample J) the incidences of *S aureus* in samples J and I were correlated In 11 cases *S aureus* was found to occur in both J and I Of these 9 had either the same phage type complex (seven cases) or were not typable (two cases) while in two cases the types of the strains were different In seven instances *S aureus* occurred only in I in 23 only in J while in one case with *S aureus* in sample I sample J was not examined Thus in 9 out of 18 cases (50 %) with *S aureus* in

Table VII

Incidence and phage type complexes of *Staphylococcus aureus* isolated on admission at the beginning and end of surgery and on the 4th and 7th postoperative days

Code	Phage type complex	Incidence of *Staphylococcus aureus*				
		Con junctiva on admission	Con junctiva begin of surgery	Wound site end of surgery	Con junctiva 4th post op day	Con junctiva 7th post op day
		Sample A	Sample E	Sample I	Sample J	Sample M
	Group I					
01	80 and/or 81	1	1		1	2
02	52 and/or 29	9	4	2	7	2
03	29/52/81					
04	52/52A/80/81					
05	Other combinations group I	8	5	4	3	
	Group II					
06	3A	3	4		3	2
07	71					
08	gr II/71					
09	Other combinations group II	7	2	2	2	2
	Group III					
10	47B and/or 47c					
11	A single type gr III					
12	Other combinations group III	4	1		1	
13	Gr III/83A/6557 (± 89)	4	3	1	2	1
14	31B/52B/6557 (± 89)					
15	Gr III/6557	1				
16	83A/6557 (± 89)	2	1			
17	Gr III/83A/(± 89)	5	2	1	1	2
18	84/85/6557					
19	84 (± 89)					
20	85 (± 89)	1				
21	84/85 (± 89)					
22	89	2	1		1	1
23	6557 (± 89)	1				
24	83A	1	1	1	1	1
27	*Group IV*					
25	*Mixed groups*	5	3	2	3	1
28	187 and/or 94	6	3	2	2	1
26	Non typable undiluted	16	6	4	7	3
99	Not (phage) examined	0	0	0	0	0
	Total	74	37	19	34	18

sample I sample J either did not show *S aureus* or had a different phage type complex The 95 % confidence limits of these figures (9 out of 18) are 26–74 %

As to the 18 strains recovered in sample M (Table VII) 10 had either the same phage type complex (nine cases) or were not typable (one case) as in A One strain (not typable) could be traced to sample I (and J) and two to sample J (originated from the patient s own nose sample B) Of the remaining five strains which were not found in A F I or J two could be traced to the patient s nose (sample B) one to the nose of a nurse while the origin of two strains could not be found

Discussion

In contrast to the preoperative conjunctival flora which has been the subject of numerous studies (Fahmy et al 1975b) relatively little has been done to investigate the nature of the postoperative flora Metafune & Albanese (1912) and Kraupa (1914) were mainly interested in the occurrence of pneumo strepto cocci on the conjunctiva postoperatively In 1954 Locatcher Khorazo & Gutier rez (cited in 1972) studied the conjunctival flora of 15 patients prior to a variety of operations and again 24 hours following surgery They observed an increase in the colony count postoperatively Furthermore in 12 instances an additional species of microorganisms was seen on the postoperative culture plate They suggested that the increase in the number of colonies was due to the well known effect of bandaging the eye (Axenfeld 1907 Linhart 1950) which promotes conditions favourable for the multiplication of bacteria This phenomena, i e increase of the colony count postoperatively has been observed by other authors (Burns et al 1968 Burns & Oden 1972) in eyes not given antibiotics postopera tively On the other hand when antibiotics were used frequently in the post operative period Burns (1959) found that the operated eye was often sterile and usually had fewer bacteria than the unoperated eye Makley & Suie (1971) studied the bacterial flora of the lids and conjunctiva before during and after intraocular surgery and found that the prophylactic use of antibiotics prior to surgery reduced the incidence of potential pathogens by approximately 60 % during surgery and by 30 % 3 days after surgery

Apart from that limited study (Makley & Suie 1971) the present series of investigations seems to be the only one in the literature in which the conjunctival flora has been studied before during and after intraocular surgery In the first part of this series the conjunctival flora was studied on admission to the hospital (Fahmy et al 1975b) and showed a typical bacterial pattern *S albus* and *coryne* bacteria predominated followed by *S aureus* gram negative bacilli and strep

tococci None of the conjunctivas was found sterile In the second part of the series when the flora was studied immediately before surgery (Fahmy et al 1975c) a significant fall in the incidence of all kinds of bacteria could be observed ($P < 0\ 0001$) This was attributed to the topical administration of antibiotics (oxytetracycline polymyxin B) 18 hours prior to surgery However 92 % of the conjunctivas examined immediately before operation proved to harbour one or more kinds of microorganisms Furthermore 61 % of the wound sites were found to be contaminated with bacteria at the conclusion of surgery This was explained by inadequate prophylactic treatment

In the present study when the flora was reexamined on the 4th and 7th post operative days before and after the administration of antibiotics respectively the course of the various kinds of bacteria proved to be different The incidence of *S albus* increased on the 4th postoperative day to the same level as that found on admission (E ~ J $P < 0\ 0001$ A ~ J $P > 0\ 05$) However when the number of colonies of *S albus* was matched on both occasions i e on admission and 4th postoperative day a significant increase could be observed postoperatively ($P = 0\ 0216$) This was probably due to the above mentioned effect of bandaging the eye Corynebacteria and gram negative bacilli likewise showed an increase in incidence postoperatively (E ~ J $P = 0\ 016$ and $0\ 0022$) but not to the same level as on admission (A ~ J $P < 0\ 0001$) The incidence of *S aureus* and streptococci on the 4th postoperative day did not vary from that found at the beginning of surgery (E ~ J $P > 0\ 05$) On the 7th postoperative day *S aureus* showed a fall in incidence ($P = 0\ 0052$) while other bacteria had largely the same incidence as that of the 4th postoperative day This is probably due to a selective effect of the antibiotics administered routinely on the 4th postopera tive day

One of the main objects of the present investigation was to assess the origin of *S aureus* found on the conjunctiva postoperatively and at the same time study the influence of some factors such as nasal flora of the nursing staff air of the wards and eye medicaments upon the occurrence of *S aureus* on the conjunctiva

The present results showed that the great majority of strains isolated post operatively were similar to those found at the time of admission In a few in stances the source of *S aureus* could be traced to the patient s own nose or to the noses of some of the nurses In no instance could *S aureus* be traced to the air of wards

In a study carried out in another surgical department (Surgical Department I Kommunehospitalet) specializing in gastro enterology Thomsen et al (1970) demonstrated that the air of the wards was of major importance concerning the route of postoperative wound infections caused by *S aureus* *S aureus* was iso

lated from 162 out of 398 (40 7 %) agar plates exposed postoperatively to the air of the wards In the present study *S aureus* was isolated in only 8 out of 463 (1 7 %) plates This is probably due to the different kinds of operations undertaken in the two departments As is known eye surgery is usually performed on clean eyes while abdominal operations are often carried out on septic cases Further it should be mentioned that is has been possible in the present study to isolate nearly all infected cases admitted during the investigation in single rooms without contaminating the air of the other wards

It is a well known fact that contaminated eye drops and ophthalmic ointments *may serve as a source of infection in ophthalmic wards* (Lindner 1914 McCulloch 1943 Rintelen 1951 King 1953 Rauh 1953 Richter 1955 Goettsch 1956 Custodis 1958 Crompton 1961 Norn & Frølund Thomsen 1967 Allen 1970 Ertel et al 1973) Smith (1954) and Ridley (1958) stated that cross infections from one conjunctiva to another may occur in the wards by means of eye drops *However little seems to be known of the epidemiological role actually played* by contaminated eye drops upon the postoperative conjunctival flora

The present study could not give an answer since *S albus* the only contaminant isolated from eye drops in the present study is not suitable yet for epidemiological methods Further *S albus* was isolated from almost all the patients postoperatively (sample J and/or M)

It is still a traditional practice among many ophthalmic surgeons to obtain cultures from the conjunctiva in cases of frank or suspected postoperative endophthalmitis No study has been made yet to determine the value of such a culture for detecting the organism which has actually entered the eye

The present study has attempted to examine the relationship of bacteria *isolated from the wound site at the end of surgery to those found on the* conjunctiva during a time when endophthalmitis was most likely to develop i e on the 4th postoperative day (Fahmy 1975) When *S aureus* was chosen as an index mainly because it is suitable for epidemiological analysis it was seen that out of 19 strains found on the wound site at the termination of surgery only nine *of a similar type were isolated from the conjunctiva on the 4th postoperative* day while in a single patient showing *S aureus* peroperatively the samples were not examined postoperatively This may demonstrate that a culture obtained on the 4th postoperative day will fail to reveal *S aureus* actually present on the wound site at the end of surgery in 26–74 % of the cases (95 % confidence limits of 9 out of 18) The same applies to most of the other bacteria (Table IV)

Consequently on the basis of these results it may be concluded that conjunctival cultures obtained to establish the etiology of postoperative endophthalmitis

are probably of little value in reflecting the flora actually present during the time when infection is most likely to occur Anterior chamber paracentesis may seem to be a better alternative (Fahmy 1975)

Acknowledgements

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RETINAL DYSTROPHY COMBINED WITH ALOPECIA

BY

AKE BJÖRK and PEDER JAHNBERG

Retinitis p gmentosa or pigmentary retinal dystrophy is the most important group among the tapeto retinal dystrophies The degenerative process found in the retina has in many cases a corresponding counterpart in other parts of the body most commonly in the CNS Combinations of retinal dystrophy and for instance deafness or mental retardation are well known Also combinations of retinal dystrophy and metabolic dyscrasia have been reported

Only few reports on the combination of retinal dystrophy and dermatological affections have been found We have found two cases of almost total alopecia combined with retinal dystrophy Since we have succeeded in finding a few similar cases in the literature we consider this apparently rare combination worth a somewhat closer analysis as it might constitute a specific entity

Key words retinal dystrophy - alopecia - progeria - ocular syndromes

The term tapeto retinal degeneration coined by Th Leber in 1916 compiles several morphologically different degenerative states which all involve the pigment epithelium giving a typical appearance to the ocular fundus

This group of conditions has evoked much interest also from a genetical point of view and its highest frequency is found in isolates They are therefore not

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uncommon in certain parts of Sweden but like in many other countries we find a decrease in the number of new cases owing to the greater mobility of the population However from an ophthalmological point of view these conditions are still important as they usually have a progressive course often giving a high degree of invalidity

In the English language literature this group of conditions is generally known as tapeto retinal dystrophies A striking feature of the group is also the large number of phenotypic differences which may occur in the same family as manifestations of the same genotypical defect This is also true both for the type of dystrophy and the age of its manifestation These considerations as well as the many transitional forms which may occur in the same pedigree strongly suggest that these dystrophies are all essentially of the same nature (Duke Elder 1967)

Retinitis pigmentosa now more often known as *a typical pigmentary dystrophy* is the most important group of the tapeto retinal dystrophies *constituting a morphologically more uniform group with peripheral atrophy of the retina typical pigmentations mainly in the periphery and a reduction of the visual field* as the most striking characteristics Night blindness is an important symptom whereas the visual acuity is preserved longer

The degenerative process found in the retina has in many cases a corresponding counterpart in other parts of the body most commonly in the CNS Retinitis pigmentosa in particular is sometimes combined with other defects such as mental retardation and deafness In the syndrome of Lawrence Moon Biedl there is a combination of retinitis pigmentosa and genital hypoplasia polydactylia obesity and oligophrenia as well as other defects Cockayne s syndrome is a rare combination of retinal dystrophy shortness of stature progeria oligophrenia and deafness

There are also other combinations Metabolic dyscrasias with very serious functional defects might be combined with retinal dystrophy There have been only a few reports on the combination of retinal dystrophy and dermatological affections We have found two cases of almost total alopecia combined with retinal dystrophy As we can also refer to similar cases in the literature we consider this apparently rare combination worth a closer analysis as the combination might constitute a specific entity

Own cases

A brother and sister from the northern part of Sweden have been submitted to thorough investigations at the Department of Ophthalmology Karolinska Hospital Stockholm

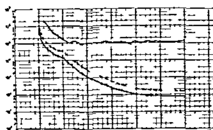


Fig 1

Case I Upper curve Dark adaptation curve showing a highly pathological course The lower unbroken curve Mean threshold values in healthy individuals in this age range Broken curve Upper border line of the normal range of threshold values

Case I

S G A woman aged 37 The third of four siblings A brother the youngest of the siblings was examined this year and showed no signs of eye disease He has a normal hair growth An elder sister is reportedly quite healthy One brother Ö S has the same disease as this patient (see below) No similar cases are known among the other relatives The families of both the mother and the father come from the northern part of Sweden near Kalix No consanguinity is known

At the age of 6 the patient s hair became thinner until almost total alopecia had developed and this condition has remained unchanged She always wears a wig

She was examined by an ophthalmologist for the first time in 1970 at the age of 35 Her visual acuity had decreased during the last few years and she thought that she needed new spectacles She was myopic and had earlier received spectacles from an optician The examination was performed at the Dept of Ophthalmology in Boden and was completed with some further investigations at the Dept of Ophthalmology Karolinska Hospital Stockholm

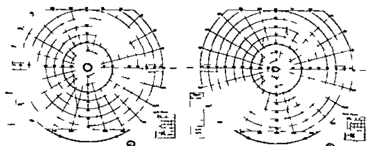


Fig 9

Case I Visual fields Highly reduced fields in both eyes

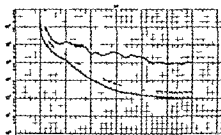


Fig 6

Case II Upper curve Dark adaptation curve showing a highly pathological course better however than that of case I The two lower curves See Fig 1

Since 1970 she has been examined at the Department of Ophthalmology Karolinska Hospital about twice a year During this time a deterioration of her vision has been noticed both subjectively and objectively The visual acuity of the right eye has scarcely diminished but in the left eye it decreased from 0 4 to 0 2

In 1970 the visual fields were very small and have since become still more reduced The visual field of the right eye today extends to about 5° around the fixation point and that of the left eye is even less (Fig 2)

Case II

U S A man aged 45 the eldest of the four siblings He had always had very scarce hair growth and this condition proceeded very early on to almost total loss of hair While still in school he had no obvious visual impairment In darkness however he experienced trouble and moved a little unsteadily A few years ago he noticed a more pronounced loss of vision he became aware of this when he knocked against furniture and other objects and sometimes details in the visual field suddenly disappeared Last year his difficulties became so severe that he consulted an ophthalmologist Apart from these discomforts the patient considers himself healthy He is unmarried and has no children He is an office employee

Examinations in the autumn of 1974 revealed the following facts

Normal body structure but a little short statured Like his sister he showed no signs of mental disturbance or reduced intellectual capacity He had almost total alopecia including the eyebrows and lashes (Fig 3) Visus on the right eye = 0 7 (-1 00 spherical combined with -0 75 cylinder 180) Visus on the left eye = 0 7 (-1 50 spherical) A very slight opacity at the posterior pole of the lens was noticed

Ocular fundi The discs were pale with a greyish tint The retinal vessels were very narrow and the entire retina was thin and atrophic with choroidal structures dominating the fundus The maculae were without marked changes (Fig 4)

Visual field examination showed large defects (Fig 5)

The dark adaptation curve was highly pathological (Fig 6) ERG was extinguished in both eyes when stimulated by light of 20 80 and 800 lux and electronic flash

Discuss...

Congenital alopecia is rare and this is p... isolated defect Hereditary factors are of ... autosomal recessive hereditary disorder has ... (Sly & Treister 1967)

Several syndromes are known in which alopecia ... or several defects which together give the individ... both physical and mental Also ophthalmologica... syndrome and in some relatively rare cases retin... generally of unspecific type Reports on these cas... regards the ophthalmological findings as it is d... vanced examination in very young or mentally d...

Furthermore examination of the ocular fundus ... ocular defects e g cataract microphthalmus and ...

Progeria or the Hutchinson Gilford syndrome is a ... with premature ageing baldness dry skin and loss of ... Total alopecia may be present Skeletal and vascular ch... the patients are dwarfs with relatively large heads ... leads to an early death in many cases from coronary ... progeria with eye defects have been reported (Gre... 1969) A closely related syndrome is Werner s syndrome ... of the adult Eye changes with retinal dystrophy ha... condition (Valero & Gellei 1960) The general appear... reminds somewhat of progeria The shortness of stature ... especially in the woman together with the alopecia are ... to this syndrome Nevertheless this diagnosis could ... many reasons The age of our patients is reason enoug... not been able to include our patients in any other known ...

Our cases are however not completely unique Of ... connection is a report from an ophthalmological clinic ... (1965) in which he describes two sisters with total al... degeneration The younger sister was 19 years of age at t... tion She had never had any hair on her head and was ... very scanty hair on other parts of the body Despite a ... acuity she managed well in school and had no difficu... years she has experienced increased reading difficulty ... creased Her visual acuity at the time of examination w... and 1/60 in the left Alternating esotropia was found ... fundus a central atrophy with pigmentations of irreg...

the macular region The periphery of the fundus was normal as were the retinal blood vessels Visual field examination was made only partially and gave the impression of a concentrically reduced field and a central scotoma Dark adaptation was normal ERG recording showed normal *b* waves but the *a* wave was three times the normal size Internal medical examination showed nothing abnormal

The older sister 26 years of age at the time of examination was essentially a parallel case She managed well in school and at that time probably had normal vision At the age of 19 her visual acuity rapidly deteriorated and was soon only 2/60 in the right eye and 1 5/60 in the left The mediae were clear Ophthalmoscopic examination of the fundi revealed large central atrophic areas with pigmentations involving the maculae and the peripapillary regions The peripheral parts were normal Retinal vessels were also normal Dark adaptation was essentially normal but the ERG recording showed significantly lowered *b* waves but a normal *a* wave No eye disorders of importance were known of among the relatives and there was no known consanguinity

Hereditary macular degeneration in three generations was described by Johnston Darragh & Nevin (19 3) In their report they described a family in which eight members in three generations had had macular degenerations Two affected brothers also had alopecia congenita One of them had a coarctation of the aorta as well and their mother who had macular degenerations also had congenital aortic stenosis The inheritance of the eye disorder was of the autosomal dominant type The authors considered the alopecia to be probably unrelated to the macular degeneration In contrast to our two patients and to the two sisters in the report from Budapest here there is a definite hereditary component as regards the retinal degeneration

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CATARACTA OSSEA AND OTHER INTRAOCULAR OSSIFICATIONS

A case report and a thirty year Danish material

BY

HANS FLEDELIUS

Cataracta ossea is described in a 5 year old Danish boy representing a sequal to a unilateral measles endophthalmitis 3 years earlier Ossification was not demonstrated elsewhere in the eye
Further cases of intraocular ossification over a 30 year period were reviewed based on the files of the Eye Pathology Institute The total of 155 made up about 3 % of enucleated eyes from Danish material received for examination Regarding age at the initial eye lesion more than half appeared within the first decade of life The most frequent underlying lesions were trauma (32 %) and uveitis (25 %) Among the less frequent causes emphasis is given to three cases of malignant uveal melanoma Ossification of the lens was not encountered in the series – except for the case which motivated the review

Key words cataracta ossea – measles endophthalmitis – intraocular ossifications – ossified choroidal melanoma – ocular trauma

Cataracta ossea – ossification of the lens – is very rare in eye pathology The literature mentions only a few cases most of which are dominated by the much more frequent choroidal ossifications of enucleated phthisical eyes

The present report deals with a) a 5 year old Danish boy presenting with cataracta ossea (an unusually clear cut case) which developed after a measles

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endophthalmitis 3 years earlier and b) a short survey is given of 155 eyes with intraocular ossifications assembled from the files of the Eye Pathology Institute over a 30 year period

Material and Methods

a) A case of cataracta ossea

Clinical history Enucleation of an amaurotic *right* eye was performed in a Danish boy aged 5 years and 9 months after 3 years of recurring episodes of ocular pain and lacrimation

The mother's pregnancy was uncomplicated with delivery at term the child's subsequent development was normal and the relevant familial history was considered negative

At the age of 2 years and 8 months he suffered from measles which was soon complicated by a severe infection of the right eye The presenting signs were swollen eyelids and purulent discharge He was admitted to hospital (Med dept Herning Sygehus) 6 days after the onset of the exanthema Upon admission his right cornea was oedematous and almost opaque but without evidence of previous or recent perforation due to injury or ulceration A plastic green yellow exudate filled the anterior chamber also making visualisation of iris and lens impossible On suspicion of an intraocular abscess he was immediately transferred to the University Eye Clinic Århus Kommunehospital During treatment with antibiotics (ampicilline parenterally chloromycetin locally) and cycloplegics (atropine sulphate eye drops) the condition gradually improved and it was possible to postpone the intended enucleation of the already blind right eye When discharged from hospital 3 weeks later the eye was quiet without injection and the cornea was transparent there was iris bombé with an almost obliterated anterior chamber A greyish membrane covered the displaced pupil and the lens could not be seen Earlier in the history however the lens had initially been described as normal and later as cataractous The optical conditions never allowed an ophthalmoscopical evaluation of the posterior eye segment

Except from the initial period of fever the boy was in a good general condition elevation of the ESR and a slight anaemia were only transitory Serologically as well as clinically there was no evidence of venereal disease Still's disease or other rheumatoid conditions

The slightly phthisical blind *right* eye was removed 3 years later (Eye dept Esbjerg Sygehus) because of irritation and cosmetic blemish The *left* eye remained unaffected during the whole clinical history with normal visual acuity

b) 155 cases of intraocular ossification a 30-year material

All cases of intraocular ossification were collected from the files of the Eye Pathology Institute covering the period 1944-1973 With only a few exceptions all specimens were seen by the Head of the Institute (S Ry Andersen) usually however in collaboration

with the senior and junior members of the staff The evaluation is regarded as uniform and reliable

The clinical history was taken from the information given on the pathology request forms whose data were usually regarded as relevant to the scope of this study

Pathology

a) (Eye Path inst no 156/74) After fixation for 24 hours in buffered 4 % formaldehyde and 1 % glutaraldehyde (pH 7 2) the eyeball was measured in three dimensions exa mined in a dissecting microscope and cut horizontally Routine paraffin technique was used (without decalcinating procedures) and deparaffinized hydrated sections were stained with haematoxylin eosin In addition the following special stains were applied P A S haematoxylin phloxine safranin Brown Brenn Grocott and Ziehl Neelsen In a later procedure serial sections were made from the rest of the eye to reveal osseous tissue outside the lens if present

b) The 155 eyeballs with bone formation had all been processed according to the laboratory s routine with an average of 10 sections examined per eyeball

Results

a) The eye of the 5 year old boy

External measurements of the eyeball 23 x 21 x 21 mm In the cut eyeball a funnel shaped total retinal detachment appeared The vitreous was milky and coagulated and whitish masses were seen in the anterior chamber Macroscopi cally a well defined lens structure was not apparent

Microscopical examination (Fig 1) showed a slightly phthisical eye with a normal cornea perilimbal zone and sclera The uveal tract appeared atrophic especially in the anterior parts but without evidence of inflammation calcifica tion or ossification and the same was true of the totally detached gliosed retina The most conspicuous changes were confined to the lens region in which the usual lens structure was replaced by an osseous tissue with typical Haversian canals In some sections small folds of lens capsule could be demonstrated be *tween the posterior part of the osseous tissue and the retrolental fibrous mem* brane which was composed partly of the atrophic detached retina The special stains added no further information bacteria or fungi could not be demon *strated in the sections*

b) The series of intraocular ossifications

1 Frequency The 155 eyes with intraocular ossification made up 3-1 % of the eyeballs from Danish material received in the Eye Pathology Institute for histopathological evaluation

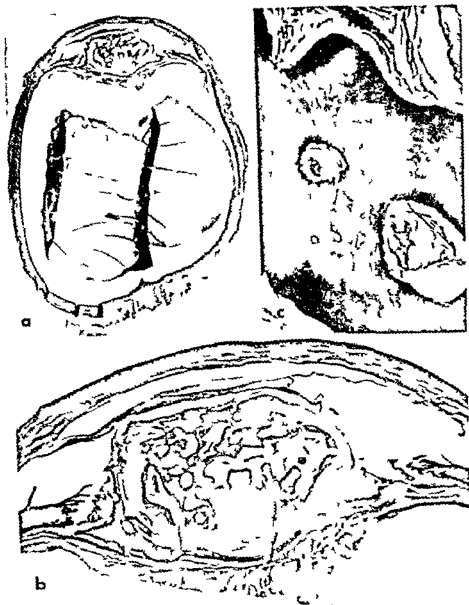


Fig 1

Histopathological sections of an eye with cataracta ossea (Eye Path Inst no 756/74)
a) Survey of the whole eye Ossification is seen only in the anterior eye segment (× 3.5)
b) The anterior eye segment with ossified lens (× 10) c) Lenticular osseous tissue with Haversian canals (× 100)

2 *Sex ratio* It was here considered relevant to subdivide the series according to origin traumatic (51)/non traumatic (104) In the group with trauma there were significantly more males (38/13) than females whereas the slight female pre ponderance (59/45) in the non traumatic sample was insignificant (χ test) This conclusion also held for the sex ratio in the whole group (83 males 72 females)

3 *Site of ossification* The degenerative osseous changes were mainly confined to uveal tissue especially the posterior part of the choroid The exception was a case of Coats disease with isolated occurrence of retinal ossifications In addition to choroidally located bone formations simultaneous occurrence was described in the retina (10 cases) the optic nervehead (one case) and the vitreous body (7 cases) *There were no cases of lenticular ossification within the series while* calcified lenses were observed in about one third of the eyeballs (54/155)

4 *The initial ocular lesion (age of patient at onset type of lesion)* Table I lists the various underlying eye disorders and their frequencies in the total group

Table I

Underlying eye disorders in 150 enucleated eyes with intraocular ossification (5/155 cases excluded due to lack of information) The series is subdivided according to the age of the patient when the initial disorder appeared

	Frequencies in the whole series n = 150	Age (years) of patient at the clinical onset of eye disease or trauma	
		Before the age of ten n = 89	After the age of ten n 69
Traumatic injury	48 = 32 %	29	19
Uveitis	37 = 25 %	16	21
Corneal disease	22 = 15 %	10	12
Buphthalmia	10 = 7 %	10	
Other congenital malformations	10 - 7 %	10	
Glaucoma (except congenital)	6	1	[illegible]
Retrolental fibroplasia	5	5	
Uveal melanoma	3		3
Retinal detachment	2		[illegible]
Sequels to eye surgery	[illegible]		
Phthisis or unspecified	5	1	4

and in subdivisions according to age at onset (before or after the age of 10 years) The largest group traumatic injury accounted for about one third of the cases Within this group 60 % followed perforation injuries

In more than half of the patients (82/150) the ocular lesion could be dated back to the first decade of life Only five cases had an indefinite onset in relation to the arbitrarily chosen division at the age of 10 years Four members of the series were even below the age of ten at the time of enucleation (a boy aged four with microophthalmia and possible trauma a boy aged five with Coats disease a girl aged six with retrolental fibroplasia and a boy aged seven with congenital lesion)

5 *Time interval up to enucleation* A painful red eye was the main reason for the enucleations performed Usually a considerable time span had elapsed from the initial lesion This period could be estimated with accuracy in 48/51 traumatic cases and in 80/104 non traumatic The median values were 24 and 23 years respectively (with the mean values a little higher 31 and 28 years) In only 10 % (13/128) were there intervals shorter than 10 years The shortest time span was 1 ½ years This occurred in an 18 year old man with a perforation injury in a previously healthy eye

Discussion

a) The above case report deals with a very clean case of cataracta ossea probably originating in a plastic exudate of the anterior eye segment accompanying a severe anterior uveitis or endophthalmitis 3 years earlier Since no remnants of the anterior lens capsule could be demonstrated microscopically it was assumed that this structure had been damaged and disintegrated in the initial acute phase of the eye disease Rupture of the lens capsule in infancy usually leads to absorption of lens material resulting in aphakia with secondary cataract In our case however the inflammatory exudate adopted the space within the capsule with subsequent organization and bone formation, roughly retaining an outline corresponding to the original lens The vascular supply presumably came from the anterior uvea by way of the retrolental membrane described above

The present case of osseous cataract is the first to be seen in this institute and to the best of our knowledge also in the whole of Denmark Michail (1934) described a Rumanian case with intraocular calcification and ossification confined to the lens choroid and retina in a 19 year old woman Michail reviewed the literature to that date and found a further three cases (aged 15 18 and 31 years) in which lenticular ossification was a predominant feature He pointed

out that the prerequisite for osseous lens changes is an ocular lesion with onset early in life and affecting predominantly the anterior eye segment Samuels (1938) described bone formation within the lenses in 7 out of 91 globes with ossification but in all seven it was combined with bone elsewhere in the eyes In more recent literature Zeiter s series (1962) comprised 46 specimens with intraocular calcification/ossification but no cases of cataracta ossea Blatt (1965) gave no details in his series of 26 globes with ossification

b) *While the few reports on cataracta ossea presumably reflect the rare occurrence of this phenomenon the same cannot be said of uveal ossifications* This more commonplace finding is however the subject of study in only a few larger series The reason for this lack of wider interest is easily explained from the pathologist s viewpoint ossification is simply a well known endpoint in states of longstanding degeneration – ocular as well as outside the ophthalmic regions Again for the clinician intraocular bone formation is confined to blind eyes in which the therapeutic problem is reduced to the alternatives of enucleation or *medical treatment if painful*

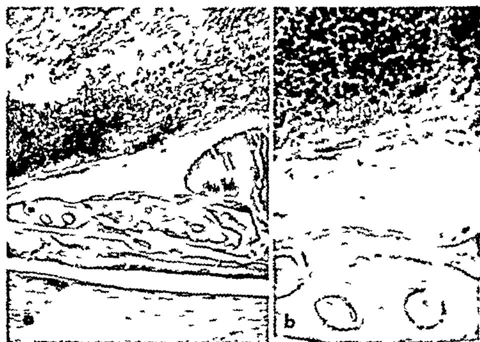


Fig 2

Histological sections from an eye with melanoma and uveal ossification (Eye Path Inst no 181/48) a) Osseous tissue is seen between the *choroidal melanoma* (above) and the sclera (below) (x 40) b) Melanoma cells and osseous tissue in higher magnification (x 100)

Zeiter (1962) however stressed some points of more general interest concerning ocular X ray shadows a) as evidence of calcification in small infants suspected for retinoblastoma and b) in relation to foreign body differential diagnosis Zeiter (1962) further stressed that two notorious causes of ocular degeneration do not lead to calcium deposition sympathetic ophthalmia and melanosarcoma This was explained at least in part by the fact that enucleation in such conditions is usually performed early in the clinical history The generally accepted lack of bone formation in eyes with uveal melanomas has been advanced as a form of evidence for the theory that necrotic eye changes are usually secondary to the melanoma and that melanomas do not often arise in eyes already phthisical because of other causes In this connection it is interesting to note in the present series three cases of ossification (out of the total 155) in eyes with verified uveal melanomas The most clear cut case is shown in Fig 2

Otherwise the present series is in general agreement with material already published and only a few further points will be mentioned As for the frequency of intraocular bone formation the actual number of 3–4 % refers only to the number of eyeballs received for histologic evalution This of course has no bearing upon the real frequency since many ossified globes remain clinically silent and therefore escape enucleation

Finally it is stressed that the calcareous changes in eyes with retinoblastomas in no instance proceeded to ossification prior to the surgical treatment. This conclusion is based upon the microscopical examination of a total of 294 eyeballs with retinoblastoma from foreign and Danish material received in the Institute over the years

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Saraux H Abrégé d'ophthalmologie 3rd ed Masson Paris 1975 204 pages 111 illustrations price D Kr 67 15

This short book on ophthalmology is intended for non ophthalmologists and students Its primary object is to give some guidelines in the management of acute cases as well as the more simple cases which can be treated without the assistance of an ophthalmologist It can also be used to brush up ophthalmological knowledge which the reader may have had but has now forgotten

The text is (by intention) brief and discussion on pathogenesis reduced to a minimum The reader is assumed to be familiar with the extraocular signs and symptoms of the general affections discussed In the preface the hope is expressed that this short concentrated style will be appreciated by those hesitating to consult a more extensive ophthalmological text The book can be recommended to general practitioners

Niels Ehlers

Jackson C R S The Eye in General Practice 7th ed Churchill Livingstone Edinburgh 1975 174 pages 48 illustrations Price £ 5 00

This useful book covers all the day to day ophthalmological problems likely to be encountered in general practice It is small and concise yet comprehensive and easily readable The book is intended not only as an introduction to ophthalmology but also as a handy and up to date reference source on the subject The commoner eye diseases are presented with good clinical descriptions of signs and symptoms and sound practical advice is offered concerning treatment Attention is drawn to the difficulties facing eye patients The use and misuse of cortisone preparations the value of proper assessment of ocular injuries and the management of cataract glaucoma and squint patients are amongst those aspects which are explained in a readily understandable manner Finally the book is characterised by many excellent colour photographs making it a valuable asset to student and practitioner alike

Martin Lowes

Bellows John G (ed) Cataract and Abnormalities of the Lens Grune & Stratton Inc, New York 1975 533 pages Price $ 36 50

This textbook deals with the many aspects of complicated and senile cataracts In the sections on composition and metabolism of the lens out of date units of measurement are used e g mg 100 g thus impeding direct comparisons with the results of other recent reports If conversion is made to meq/kg lens for Na and K+ from mg/kg as stated in this book one will find that these two important ions are represented in identical quantities in human (clear ?) lenses This condition is stated to be representative which is questionable and in contrast with the findings of certain other authors Also in places only reference is made to human metabolite concentrations while results of animal

investigations are quoted The section on laser holography opens new aspects regarding the diagnosis and optical correction of even small lens opacities which may cause visual distortion Mutations free radicals and molecular crosslinking may be responsible for ageing and cataractogenesis A good description of electron microscopic findings is given and such information is hard to find in textbooks Phaco emulsifica tion Barraquer s keratophakia as a method for correcting large ametropias intraocular lenses Girards new scleral expander and its possibilities as well as ordinary operative procedures and postoperative complications are dealt with at length

On the whole this book, written in comprehensible style, is a valuable guide for any one taking an interest in cataract The book comprises pretty well our present knowledge on this fascinating subject which in this book in certain sections is presented as a visible drama of the life and death of an organ

A Bruun Laursen

Saunders L Z and *Rubin L F* (Philadelphia Pa) Ophthalmic Pathology of Animals An Atlas and Reference Book XIV + 238 p 27 Figs 114 Plates S Karger Basel 1975 bound SFr 163 / DM 155 / US $ 68

This is the first textbook of veterinary ophthalmic pathology It is dedicated to Sir Stewart Duke Elder because of the inspiration information and enrichment of our professional lives which his monumental System of Ophthalmology has brought to us

The first 13 pages describe the very fascinating history of veterinary ophthalmic pathology showing the deep influence on veterinary ophthalmology and ophthalmic pathology of particularly human ophthalmologists In the references placed after each disease the names of many well known now living human ophthalmologists and ophthalmic pathologists are met and following the charming way of Duke Elder n his System many sections of the book are opened with an illustrated biographical sketch

The book is made such that the text is on the left page and the figures illustrating the disease concerned are on the right arranged into a plate In this way diseases of the cornea uvea lens vitreous retina optic nerve eye as a whole neoplasms and adnexal lesions are treated. The text is short but sufficient and the black/white figures including several electron micrographs are illustrative and technically ex cellent A very great advantage is the thorough comparison between human and animal conditions For a human ophthalmic pathologist it is very interesting to meet a lot of well known entities On the other hand many diseases are unknown to him

At the end of the book is a chapter on postmortem technique and histological prep aration, very valuable for all studying eyes histologically This book is a must for all human and veterinary ophthalmic pathologists and veterinarians and should be found in all laboratories using experimental animals The modest sentence of the authors – we hope that the readers will show forbearance for the efforts of two dilettants whose enthusiasm exceeds the amount of time they have to cater to it is put to shame after going through this atlas and text and the reviewer has no doubt that com ng editions like Hogan and Zimmerman s ophthalmic pathology will flourish into a two or more volume work

O A Jensen

VARIA

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University Eye Department (Head Prof Thore Lie Thomassen)
Rikshospitalet Oslo Norway

THE DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED PEROXIDASE IN THE OPTIC NERVE HEAD OF RABBIT AND MONKEY

BY

TOR FLAGE

Peroxidase was used as an histologic tracer to study the permeability properties of the tissues in the optic nerve head of rabbit and monkey The distribution of the tracer was observed by light microscopy Within a short time after intravenous injection peroxidase was located extra vascularly in the optic nerve head The present study confirms earlier reports of a defect in the blood optic nerve barrier in the region of the optic nerve head The tracer was found to have reached the connective tissue of the optic nerve head and to a lesser degree the optic nerve tissue proper The observations made it probable that peroxidase had reached the connective tissue of the optic nerve head by diffusion from the perineural choroid and sclera

Key words Optic nerve head – peroxidase – blood optic nerve barrier – connective tissue – rabbit – monkey

Several authors have described a defect in the blood optic nerve barrier in the region of the optic nerve head (Olsson & Kristensson 1973 Tsukahara et al 1973 Grayson & Laties 1971) The present study was initiated to investigate this observation further Horseradish peroxidase (Graham & Karnovsky 1966 Karnovsky 1967) was used as an histologic tracer in light and electron micro

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scopic studies on rabbit and monkey eyes The present article will describe the observations made by light microscopy

Material and Methods

Eyes from 9 albino rabbits and 3 vervet monkeys (Cercopithecus aethiops) were used in this study Commercial horseradish peroxidase (Sigma type II) dissolved in sterile Ringer solution was administered by intravenous injection The rabbits were adult specimens their weight ranged between 2 3 and 4 7 kg Seven rabbits were injected with peroxidase in various doses ranging from 35 to 230 mg/kg body weight Two monkeys weighing 3 8 and 4 2 kg were given 450 mg peroxidase The time from the beginning of the intravenous injection of peroxidase until the opened eye was immersed in ice cooled fixative is designated the exposure time to peroxidase The rabbits were killed by decapitation the monkeys by an overdose of barbiturate The exposure time to peroxidase for the rabbit eyes ranged between 4 5 and 120 min In the 2 rabbits with the longest exposure time that is 60 and 120 min peroxidase was given in divided doses in an effort to keep the serum level at maximum during the whole period The exposure time to peroxidase for the 2 monkeys was 60 min Again the peroxidase was given in divided doses One rabbit with exposure time to peroxidase 10 min was given an injection of antihistamine (dexchlorpheniramine Polaramin Schering) and a serotonin antagonist (methysergide Deseril Sandoz) intravenously in a dose of 1 mg/kg body weight 10 min prior to the injection of peroxidase Eyes from 2 rabbits and 1 monkey that were not injected with peroxidase were used for control studies

Immediately after death the eyes were enucleated opened at the equator and immersed in ice cooled fixative For fixation 2 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7 4 was used Approximately 15 min after the start of fixation the eyes were cut in halves at the equator and the fixation of the posterior halves continued for 2 h The tissues were washed overnight in cooled 0 1 M phosphate buffer pH 7 4 with 5 % sucrose Sections 20 to 40 μm thick were cut on a freezing microtome After washing in cooled distilled water the sections were incubated at room temperature for 15 min in tris HCl buffered diaminobenzidine H_2O_2 solution pH 7 6 Following a brief washing the sections were mounted directly on slides and examined without further staining

Control studies were made in sections from the peroxidase injected animals were the incubation in diaminobenzidine was omitted and in sections from the uninjected animals with and without incubation with diaminobenzidine



Fig 1

Rabbit optic nerve head Distinct peroxidase reaction product staining in the vessels and the perivascular connective tissue The white double arrow points to the heavily stained choroid and sclera. The small black arrows point to sensory retina ×65

Results

The terminology suggested by Hayreh (Hayreh 1972) will be used when describing the observations in the different parts of the optic nerve head

All the sections from the peroxidase injected animals showed a distinct pattern of peroxidase reaction product staining The observations were essentially the same in both rabbit and monkey eyes (Figs 1 and 2) In the sections from the rabbit eyes with short exposure time to peroxidase that is 4 5 and 6 5 min respectively the complete pattern of staining had not developed



Fig 2
Anterior part of monkey optic nerve The big straight arrow points to lamina cribrosa with distinct peroxidase reaction product staining Small arrows Sensory retina The arched arrow marks the position of the area seen in the inset ×89 Inset White arrow points to the heavily stained choroid the small arched arrows to vessels with stained perivascular connective tissue in the laminar and prelaminar region of the optic nerve head ×149

and the findings in these eyes will be described separately In the sections from all the other eyes there were only small variations in the staining that could be related to the different exposure times There was no distinct difference in the observations that could be related to the variations in dosage of peroxidase The choroid the vessels of the optic nerve and retina and the sclera showed strong peroxidase reaction product staining There was distinct staining of the lamina cribrosa and a moderate staining of the perivascular connective tissue in the prelaminar region A diffuse staining was seen in the optic nerve tissue proper near the perineural choroid and sclera i e in the prelaminar laminar and retrolaminar parts of the optic nerve head This staining grew heavier with increasing exposure time to peroxidase

The staining of the lamina cribrosa consisted of the staining of the vessels

and the staining of the connective tissue In higher magnification the stained connective tissue of the lamina cribrosa showed small projections into the surrounding optic nerve tissue (Fig 3) Apart from the areas with diffuse staining mentioned above the sensory retina and optic nerve tissue were not stained

The sections from the eyes with short exposure time to peroxidase also showed strong staining of the vessels and in the choroid The sclera was only partly stained The connective tissue in the lamina cribrosa and in the prelaminar region was moderately stained in some parts and virtually unstained in other parts (Fig 4 A) Detailed observations showed that the intensity of staining did not depend on the presence of small vessels in the connective tissue strands but seemed to vary with the degree of staining of the surrounding choroid and sclera (Fig 4 B) Controls The sections from the animals that had not received peroxidase showed endogenous peroxidase activity in

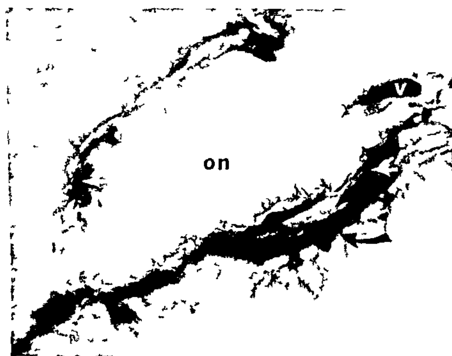


Fig 3

Detail of rabbit optic nerve head The white arrows and the v mark small vessels which are heavily stained with peroxidase reaction product The arched arrow points to stained connective tissue with small extensions into the surrounding optic nerve tissue × 930



Fig. 4

A. Rabbit optic nerve head. Exposure time to peroxidase 6.5 min. The white arrow points to the heavily stained choroid. The small arrow points to the sensory retina. The black rectangle marks the part of the section seen in greater detail in B. ×44. B. scl: sclera partly stained with peroxidase reaction product. on: optic nerve tissue. Arched arrow points to vessel with stained perivascular connective tissue. Straight arrow points to vessels with unstained perivascular connective tissue. ×179.

the erythrocytes, but no further staining. In the sections from peroxidase injected animals where the tissues had not been incubated in diaminobenzidine there was no peroxidase reaction product staining at all.

The distribution of peroxidase reaction product staining in the sections from the animal that received antihistamine and serotonin antagonist prior to the injection of peroxidase was the same as in the rest of the material. Cotran & Karnovsky (1967) have shown that peroxidase itself may alter the perme-

ability of certain tissues in some species They also showed that antihistamine and a serotonin antagonist given in combination prior to the injection of per oxidase would eliminate this effect

Discussion

The results of the present study confirms earlier reports of a defect in the blood optic nerve barrier in the region of the optic nerve head Following intravenous administration peroxidase is located extravascularly in this region in rabbit and monkey By light microscopy the tracer is distributed in a distinct pattern in the tissues This distribution pattern has not been demonstrated or discussed before

The structure of the optic nerve head is principally the same in human monkey and many subprimate species (Anderson & Hoyt 1969) In the present study the distribution of peroxidase to the different tissue components in the optic nerve head region is the same in the two species studied The discre pancies in the gross distribution pattern are due to the difference in organi zation of the optic nerve head in rabbit and monkey

The main feature of the distribution pattern is the localization of peroxidase to the connective tissue in the optic nerve head In the superficial nerve fiber layer and in the prelaminar region the only connective tissue present is that accompanying the vessels (Anderson 1969) The small vessels of the prelaminar region originate in part from the perineural choroid (Hayreh 1974) The lamina cribrosa consists mainly of fenestrated sheets of connective tissue continuous with the surrounding sclera Thus the connective tissue in the optic nerve head has close connection with the connective tissue in the choroid and sclera

The present study does not give evidence as to how peroxidase has reached the connective tissue of the optic nerve head but the observations made in the short exposure eyes indicate that diffusion from the tissues in the immediate vicinity i e perineural choroid and sclera is the most likely route That per oxidase should have leaked out of the vessels in the optic nerve head is much less probable since these vessels have been reported to have an effective blood optic nerve barrier to various tracers including peroxidase (Peyman & Apple 1972)

Beside the localization to the connective tissue the present study demon strates that peroxidase after intravenous administration reaches the optic nerve tissue proper near the perineural choroid and sclera This finding will be sub ject to further studies by light and electron microscopy (Flage 1975)

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THE PSEUDO EXFOLIATION SYNDROME
A SCANNING ELECTRON MICROSCOPIC STUDY

I The anterior lens surface

BY

MARTIN DAVANGER

The distribution and the surface of pseudo exfoliation (PE) material on the anterior lens surface has been studied by scanning electron microscopy The lenses were fixed in glutaraldehyde and OsO_4 and dried by the critical point method The PE granules of the peripheral band may rest upon a basal lamella which may terminate in a sharp edge bordering the intermediate zone The basal lamella may continue peripherally beyond the granular peripheral band towards the lens equator where the basal lamella may cover the zonular attachment zone The central disc is considered to be analogous to this lamella
In high magnification it is found that the surface of the granular and the lamellar PE material is formed by an irregular meshwork of tortuous fibrils which may be coiled as spirals The diameter of the fibrils is between 500 and 1000 Å and their length may be up to [illegible] µm The possibility that these fibrils are a condensation product of mucopolysaccharides is discussed

Key words pseudo exfoliation – lens – scanning electron microscopy mucopolysaccharide

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An important aspect of the pseudo exfoliation (PE) syndrome is the PE material distributed in a characteristic way on the anterior lens surface The surface details of the PE material and its distribution on the lens naturally lends itself to a study with the scanning electron microscope (SEM) By this method the distribution of the PE material may be studied and photographed in more detail



Fig 1

The distribution of PE material on the anterior lens surface The peripheral band terminates abruptly in the central direction PE material on the zonular attachment fibrils The area indicated by angles is seen in higher magnification in Fig 3 x 6? bar 0 1 mm

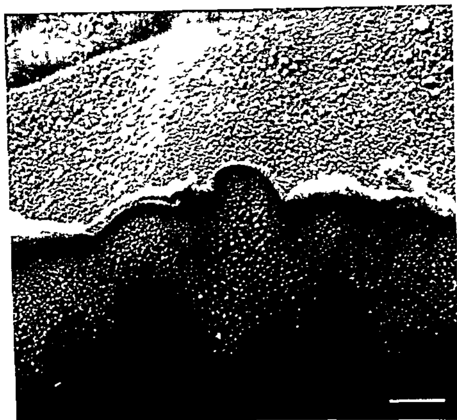


Fig 2

The basal lamella of the peripheral band terminates in a sharp edge which is partly curled up The secondary granular zone × 140 bar = 0 1 mm

than it is possible by *in vivo* examination and by incident light microscopy *in vitro* Further the details of the surface of the PE material itself may be studied in higher magnification than it is possible with any other method

Lenses with PE dried in air have been studied by SEM by Benedikt (1973) and Benedikt et al (1973a b)

In a preliminary study air drying and critical point drying (Smith & Finke 1972 Worthen & Wickham 1972) of lenses with PE was compared It was shown that in the specimens dried by critical point interesting details were present which were lost during drying in air

Results of a SEM examination of lenses with PE dried by the critical point method are presented in this paper

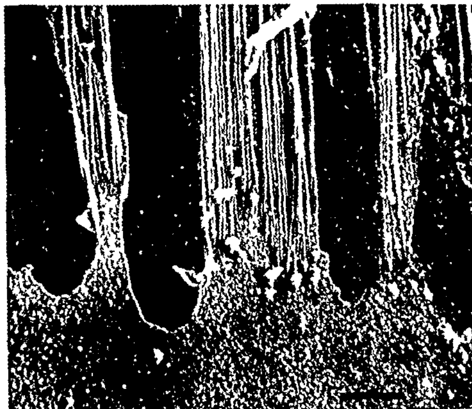


Fig 3
The peripheral part of the basal lamella continues peripherally over sectors of zonular attachment fibrils A sharp edge of the lamella borders radial zones of the lens capsule × 310 bar = 50 μm

Material and Methods

Five cataractous lenses with PF obtained by cataract cryo extraction were immediately prefixed for 2 hours in 2 5 % gluta aldehyde in 0 1 M phosphate buffer pH 7 4 After rinsing in isotonic buffer the lenses were postfixed for 2 hours in 1 % OsO_4 in Millonig s phosphate buffer pH 7 4 and dehydrated in graded acetone solutions Thereafter they were dried by the critical point method in fluid CO (Sorvall Critical point drying system)

The whole lenses were mounted on specimen holders and coated with a thin layer of carbon and gold palladium in a Edward s vacuum coating unit Scanning electron microscopy was performed with a Jeol JSM 50 SEM

That part of the lens surface which had been frozen and deformed during the lens cryo extraction was not involved in the examination

When a representative spot for a micrograph in high magnification was found a consecutive series of micrographs of gradually lower magnification (by a factor of 10 or less) were taken with exactly the same centering on the specimen Thereby the localization on the lens surface of all micrographs is recorded and can be exactly determined

Results

The distribution of PE material on the anterior lens surface is shown in the Figs 1 and 2 The PE granules of the peripheral band rest upon a basal lamella which ends abruptly in the central direction by an edge which is partly curled up (Fig 2) This edge demarcates the intermediate zone were the lens capsule



Fig 4

The edge of the central disc (CD) is partly curled up Scattered PE granules and non granular PE material are seen immediately peripheral to the edge of the central disc x 372 bar = 50 μm

seemed to be mainly uncovered Scattered PE granules however were found to attach directly on the lens capsule adjacent to the central termination of the peripheral band (Fig 2) This distribution of PE granules was described by Hørven (1935) and the area was called the secondary granular zone by Sunde (1956)

The PE granules were usually largest in the central part of the peripheral band their size was gradually reduced in the peripheral direction where the granular peripheral band was found to terminate in the characteristic tongue shaped projections directed peripherally (Fig 1)

The basal lamella to which the PE granules of the peripheral band were attached was found to continue peripherally beyond the granular peripheral band (Figs 1 and 3) In the pre equatorial region this lamella was found to cover the zonular attachment zone with the radially directed zonular attachment fibrils In sectors where these fibrils were lacking the lens capsule was not covered by the basal lamella which terminated in a sharp edge bordering the uncovered sectors of the lens capsule (Fig 3)

Typical PE granules were found to reappear in the peripheral part of the zonular attachment zone where they tended to be arranged in radial stripes following the fibrillar attachment of the zonules

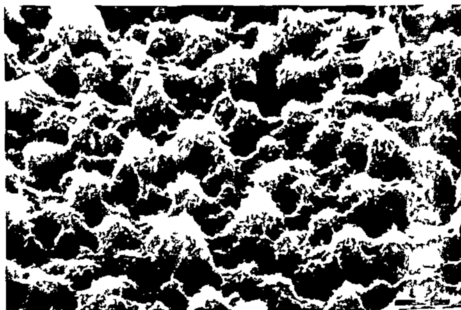


Fig 5

Typical PE granules in the middle part of the peripheral band × 1390 bar = 10 μm

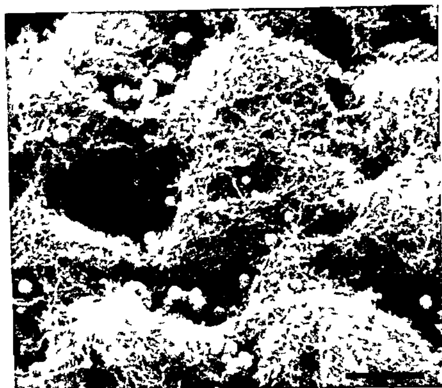


Fig 6

PE granules of the peripheral band The PE material has a fibrillar surface The fibrils may form regular spirals Spherical pigment granules ×3930 bar = 5 μm

The central disc was found to have a surface similar to that of the basal lamella peripheral to the granular peripheral band The disc was demarcated by an edge which usually was curled up in the same way as the central edge of the peripheral band (Fig 4)

Scattered PE granules as well as thin deposits of a material similar to that forming the surface of the central disc were frequently found in the inter mediate zone immediately peripheral to the edge of the central disc (Fig 4)

The PE granules of the peripheral band were found to have an irregular distribution on the surface (Fig 5) The individual granules were clearly dis cernible although neighboring granules were found to partly coalesce at places where granules were crowded The granules were irregularly spherical in shape more regularly spherical at places where the granules were smaller and

more scattered In the middle part of the peripheral band the granules were found to have a diameter in the range of 6–10 μm

In the peripheral band the lens capsule seemed to be covered completely by PE material also between the granules (Figs 5 and 6)

Spherical bodies with a diameter of ½–1 μm were frequently found on the surface of the PE material (Figs 6 and 7) Obviously they represent pigment granules liberated from the epithelial cells of the iris or the ciliary body possibly during the lens extraction

Higher magnifications revealed that the material forming the surface of the PE granules consisted of fibrils (Figs 6 and 7) Similar but not quite identical fibrils were found on the surface of the basal lamella peripheral to the granular peripheral band (Fig 8) and also on the central disc (Fig 9)

The fibrils were loosely woven together in an irregular meshwork with open spaces between the fibrils No distinct pattern could be traced in the arrangement of the fibrils relative to each other As far as it could be seen the fibrils did not branch

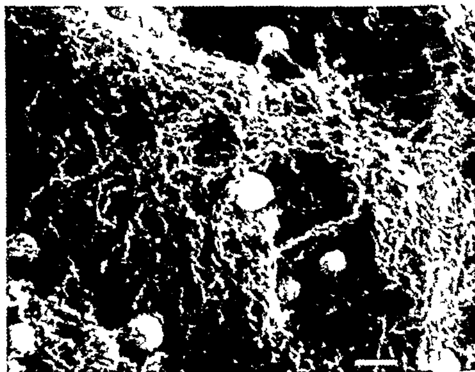


Fig 7
The surface of the PE granules is formed by a meshwork of fibrils which may form regular spirals Pigment granules × 10 500 bar = 1 μm

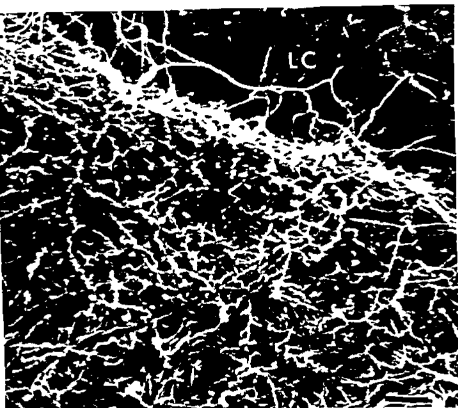


Fig 8
The surface of the peripheral basal lamella at its edge to an uncovered zone of the lens capsule (LC) as seen in Figs 1 and 3 A meshwork of fibrils x 10 500 bar = 1 μm

The length of the fibrils was difficult to determine because of their irregular arrangement it could however exceed 5 μm Their diameter was found to be in the range 500–800 Å In the average the fibrils of the basal lamella were thinner than those of the PE granules

A characteristic feature of the fibrils on the surface of the PE material was their tortuousity Most of them were more or less bent along their whole length The fibrils of the basal lamella were generally less tortous than those of the PE granules Many fibrils especially those of the basal lamella were irregularly bent But most of them were coiled to form regular spirals whose outer diameter was about 1/6 μm the distance between neighboring coils being about 1/3 μm

At some sites a faint crossbanding of the fibrils could be traced the period of which was about 200–300 Å No other special characteristics were found on the fibrils

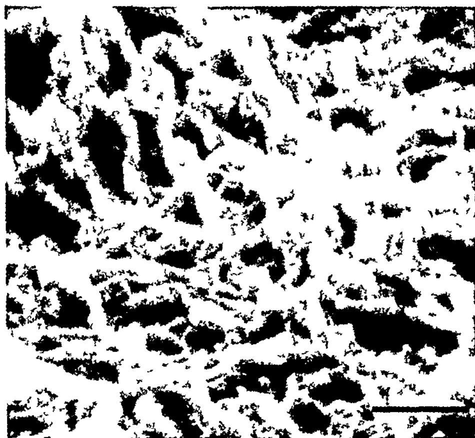


Fig 9
The surface of the central disc An *irregular meshwork of fibrils* × 49 500 bar = 0 5 μm

Discussion

The most important points emerging from this study seem to be the following

1 The basal lamella seems to be a typical and characteristic element of the PE material on the lens capsule

2 The surface of the PE material on the lens consists of a meshwork of fine fibrils

The basal lamella seems to form the base to which the PE granules are directly attached The edge of the lamella may be detached from the lens capsule as it is seen in Fig 2 and as it has been described earlier by several authors using different techniques of examination (e g Sunde 1956 Davanger & Pedersen 1975)

The basal lamella extends peripherally beyond the peripheral termination of the granular peripheral band up to the lens equator Its posterior border has not been determined

The central disc is considered to be a part of the basal lamella and analogous to this lamella in the area peripheral to the granular peripheral band

The basal lamella is difficult to recognize by slit lamp examination if its edge is not seen It is also difficult to see by light microscopy if special staining procedures are not applied (Davanger & Pedersen 1975)

The lamella is not present in the intermediate zone which is bordered by the edge of the central disc and the edge of the peripheral band The absence of the basal lamella in this region may be explained by the close contact between the lens surface and the iris (Benedikt 1973) and/or by the movements of the pupillary border over this zone (Sunde 1956) Bertelsen (1966) has described a case with congenital coloboma and PE in which an extension of the central disc continued towards the periphery in the sector of the coloboma

The absence of the basal lamella in the radial sectors of the zonular attachment zone (Figs 1 and 3) is probably an artifact caused by the zonular fibrils being removed from these sectors during the lens extraction

The fibrillar surface of the PE material represent a striking characteristic of this material The question of the nature of these fibrils is interesting and important

In a recent paper (Davanger & Pedersen 1975) evidence has been presented indicating that the PE material may be considered as composed by two main elements *protein fibrils* (seen by transmission electron microscopy TEM) are embedded in a *ground substance* considered to be mainly mucopolysaccharides

Then it seems likely to consider the following two possibilities concerning the nature of the surface fibrils of the PE material

1 They may be identical with the well known PE fibrils seen by transmission electron microscopy

Or 2 They may be formed by the mucopolysaccharides of the ground substance of the PE material possibly during the fixation and drying procedure

The thickness of the fibrils seen by SEM (the SEM fibrils) fits reasonably well with that of the TEM fibrils (when allowed for a carbon and gold palladium coating whose thickness is not determined) However their length seems to be longer than that described for the TEM fibrils (up to 1 2 μm (Bertelsen et al 1964 Ashton et al 1965 Ringvold 1969) 0 5 to 1 5 μm (Benedikt 1973)) The TEM fibrils are usually described as straight or slightly bent while the SEM fibrils usually are markedly twisted partly into regular spirals

The molecules of acid mucopolysaccharides or proteoglycosaminoglycans

are described as carbohydrate chains linked to a protein core The molecules may have a considerable length

It is suggested that the ground substance of the PE material after fixation and drying may have a fibrillar and coiled structure as that seen on the surface of the PE material

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THE PSEUDO EXFOLIATION SYNDROME
A SCANNING ELECTRON MICROSCOPIC STUDY
II The posterior chamber region

BY

MARTIN DAVANGER

The distribution of pseudo exfoliation (PE) material as well as the finer details of its surface has been studied by scanning electron microscopy on the surfaces facing the posterior chamber The specimens were fixed in glutaraldehyde and OsO_4 and dried by the critical point method after careful dissection of the anterior hyaloid surface. Granules and plaques of PE material were found to attach to the iris to the ridges of the ciliary processes to the zonules and along radial stripes on the anterior hyaloid surface one for each zonule touching this surface It seems as if the PE material is located mainly on sites of contact between two surfaces

In high magnification it is found that the surface of the PE material is formed by an irregular meshwork of fibrils the diameter of which is about 500–700 Å The fibrils are characteristically coiled, partly into regular spirals The hypothesis is put forward that these fibrils are formed by a condensation of mucopolysaccharides forming the ground substance of the PE material

Key words pseudo exfoliation – scanning electron microscopy – posterior chamber – aqueous – zonules of Zinn – anterior hyaloid – ciliary body – iris – mucopolysaccharide

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works mentioned (Davanger 1975a b) have formed the basis for the present study of the distribution of the PE material in the posterior chamber region and of the surface structure of this material

Material and Methods

The anterior segment of 3 human eyes with PE have been examined Two of these eyes were enucleated because of absolute painful glaucoma while the third was removed because of a malignant melanoma not affecting the anterior segment The methods of preparation are described in earlier works (Davanger 1975a b) The careful dissection of the anterior part of the vitreous was per



Fig 3

A conglomerate of PE granular material attached to the surface of the ridge of a ciliary process This area is also seen in Fig 2 × 325 bar = 50 μm

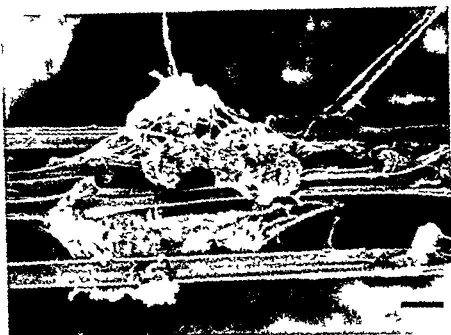


Fig 4

A group of zonules is enclosed in an accumulation of PE material Thin strands enmesh the zonules and the PE material × 1075 bar – 10 μm

formed after dehydration while the specimens were suspended in acetone The specimens were dried by the critical point method by which deformation of the specimens were avoided without the application of epon as described in earlier work (Davanger 1975a)

The ciliary body the zonules and the anterior surface of the vitreous was also photographed by incident light microscopy while the specimens were submerged in acetone

Results

Light microscopy Granules of PE material were found to adhere to the zonules which sometimes were completely covered (Fig 1 A) Granules of PE were also found to adhere to the anterior hyaloid surface in radial stripes one for each zonule which touched this surface (Fig 1 B) This phenomenon is also described by Sunde (1956) The radial stripes of PE material were several times broader than the diameter of the zonules

During the dissection of the specimens under a dissecting microscope it was observed that the PE material might anchor the zonules to the anterior hyaloid membrane Sometimes the PE material was removed from the zonules or from parts of them during the process of lifting off the hyaloid membrane from the zonules

Scanning electron microscopy A general view of the ciliary body and the zonules in a case of PE is given in Fig 2 Plaques of PE material are seen to be attached to parts of the surface of the ciliary processes preferrably to the most prominent part of the ridges Smaller accumulations of PE material were found to be attached to the zonules In the case demonstrated in Fig 2



Fig 5
PE material attached to the anterior hyaloid surface in continuous radial stripes in the zone of contact with the zonules Grooves (between arrows) run along the ridge of these stripes Area indicate by angles is seen in higher magnification in Fig 6
× 190 bar ~ 0 1 mm



Fig 6

PE material on the anterior hyaloid membrane This area is seen also in Fig 5 A groove (between arrows) along the ridge of the radial stripe is covered with thin longitudinally running filaments The PE granules indicated by right angles are seen in higher magnification in Fig 7 × 1430 bar – 10 μm

however most of the PE material followed the anterior hyaloid during the dissection and was removed from the zonules

A cobweb like net consisting of thin strands is seen to be suspended between the zonules (Fig 2)

The plaques of PE material attached to the ridges of the ciliary processes were found to consist of a conglomerate of PE granules whose diameter was in the range of 5–10 μm (Fig 3) The granules frequently coalesced into larger lumps The material seemed to be anchored upon the surface of the ciliary

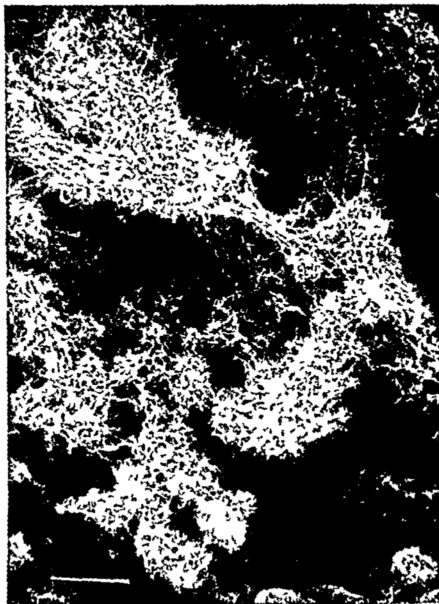


Fig 7

PE material on the anterior hyaloid The surface is formed by a meshwork of fibrils which are coiled to make spirals ×3790 bar = 5 μm

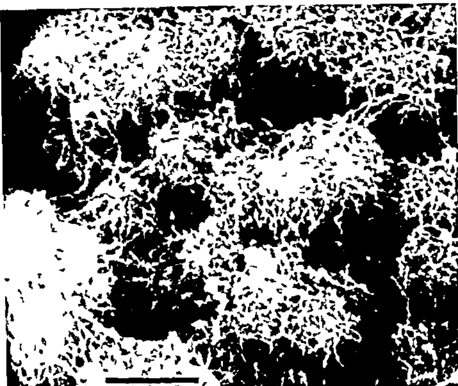


Fig 8
Fibrillar surface of PE granules on a ciliary process ×4740 bar = 5 μm

processes the material being sharply demarcated and clearly protruding above the epithelial surface This surface and also the zonules appeared normal at those areas which were not covered by the PE material

PE material attached to zonules is seen in more detail in Fig 4 A group of zonules is enclosed in an irregular mass in which however small PE granules may be recognized Numerous thin cobweb like strands most of them straight but some curled enmesh the zonules and the PE material (Fig 4)

The anterior row of zonules was usually found to be enveloped in irregular masses of PE material Thereby large groups of zonules were completely hidden for a part of their course

The PE material attached to the anterior hyaloid surface was found to be arranged in radially directed stripes (Fig 5) as it was found also by light microscopy In the case demonstrated in Fig 5 the stripes of PE material are

continuous the surface is amorphous and PE granules are not easily seen at this magnification

A groove was frequently seen to run along the ridges of the continuous PE stripes on the anterior hyaloid surface (Fig 5) The surface of these grooves was found to be covered by thin longitudinally running filaments (Fig 6) Obviously the groove represents the cast of a zonule which has been lifted off during the preparation of the specimen

The accumulations of PE material on the anterior hyaloid were found to be sharply demarcated and to protrude above the surface of the membrane which seemed to be normal in those areas which were not covered

On the posterior surface of the iris plaques consisting of clusters of typical PE granules were found The diameter of the granules was in the range 5–10 μm

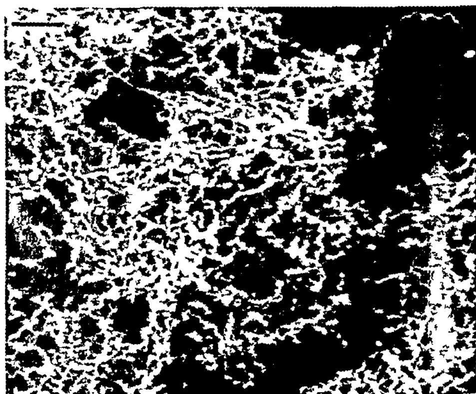


Fig 9

Coiled fibrils on the surface of PE material on the anterior hyaloid Some regular spirals are seen × 11 930 bar = 1 μm

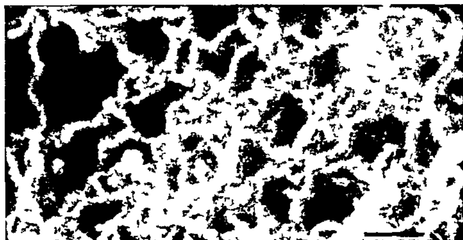


Fig 10
Coiled fibrils with spirals on the surface of PE material on the anterior hyaloid
× 29 500 bar = 0 5 μm

At higher magnifications (Figs 7 8 9 and 10) it could be seen that the surface of the PE material consisted of an irregular meshwork of fibrils with the same characteristics as it has been described for the PE material on the anterior lens surface (Davanger 1975b) At the higher magnifications the surface of the PE material was found to be about the same or similar at all the locations studied in this work.

The fibrils were characteristically coiled into more or less regular spirals The dimensions of the fibrils and the spirals were found to be similar to that described for the PE material on the anterior lens surface (Davanger 1975b) The diameter of the fibrils was found to be in the range 500–750 Å their length often exceeded 4 μm The outer diameter of the spiral was found to be about 1/6 μm and the distance between neighboring loops has been measured to ca 1/3 μm

Discussion

The PE material of the posterior chamber region has a surface which is similar to that found for the PE material on the anterior lens capsule (Davanger 1975b) In moderate magnifications *PE granules* are found similar to those

of the peripheral band on the lens And in higher magnifications the same *meshwork of coiled fibrils* can be demonstrated

The nature of these fibrils is discussed in a recent paper (Davanger 1975b) It can not be excluded that the SEM fibrils described are formed by the protein fibrils well known from transmission electron microscopy Another possibility however *is that they represent a condensation product of mucopoly* saccharides proteoglycosaminoglycans forming the ground substance of the PE material

The distribution of the PE material may help to throw some light on its formation The *surfaces of the anterior eye to which the PE material is* attached are of widely different nature (anterior lens capsule iris ciliary body zonules and the anterior hyaloid surface) These surfaces have in common that they are facing the aqueous humour Non of the surfaces consist of cell membranes (the epithelium of the iris and the ciliary body being covered by a basement membrane facing the aqueous humour)

Further it seems as if the material is located mainly on sites of contact *between two surfaces between which slight relative movements are possible* Most striking is the accumulation of PE material along the lines of contact between the zonules and the anterior hyaloid surface Similarly the distribution of PE material on the anterior lens surface is evidently influenced by *the contact of this surface with the iris* (Sunde 1956 Benedikt 1973 Benedikt et al 1973a b Davanger 1975b) Also the conglomerates of PE material attached to the ridges of the ciliary processes seem to be located on sites of contact with the anterior hyaloid

The distribution of the PE material on different surfaces of the anterior eye as well as the surface details of the material itself is best understood on the assumption that the material consists of deposits formed by a condensation *and/or an accumulation of substances from the aqueous*

The present work does not give any positive information about the original source of these substances

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DOUBLE POINT TEST WITH THE GOLDMANN PERIMETER

BY

PAUL ENOKSSON and BJÖRN FRISTRÖM

A simple accessory to the Goldmann perimeter permitting simultaneous projection of two targets is described The method can be used as a colour saturation test but also for the study of inattention hemianopia Two cases illustrate its clinical application

Key words perimetry – visual field – inattention hemianopia – diagnostic technique

A kinetic quantitative perimetry with white targets is well suited for routine use in neuro ophthalmological work (Enoksson 1965) The colour saturation test cannot replace this technique but may be a valuable complement e g when chiasmal compression is suspected (Chamlin Davidoff & Feiring 1955 Enoksson 1965 Frisen 1973 Traquair 1949) For the study of inattention hemianopia we still lack an easily applicable technique

By means of a simple accessory to the Goldmann perimeter this excellent instrument can be used both in the colour saturation test and in the search for inattention hemianopia*

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* This method was demonstrated at the meeting of the Swedish Ophthalmological Society on August 31 1974

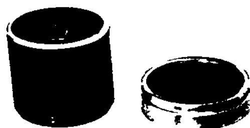


Fig 1
Prismatic device and colour filter

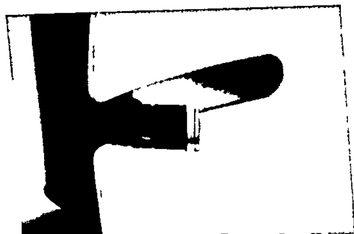


Fig 2
Prismatic device mounted on the projector of the Goldmann perimeter

Material and Methods

The new accessory consists of a small tube which contains two prisms (base out) and is fitted to the end of the projector in the Goldmann perimeter (Figs 1 and 2) Two different devices were used one with 4 diopters and the other with 8 diopters prisms A Kodak Wratten filter no 29 was added in the colour saturation test

Two elliptical white or coloured targets can be projected onto every re quired spot of the visual field horizontally vertically or obliquely In the colour saturation test the targets were projected symmetrically on either side of the vertical meridian and the patient was requested to report any difference in colour The same type of projection was used in the study of inattention hemianopia and the patient was questioned about the number of targets he could see If he perceived both he was asked about differences in brightness or colour saturation

As the projection distance differs for the two targets these are not identical in colour or size but the differences are slight and presumably without real clinical importance Anyhow the error can be checked by a repeat examina tion with the projector arm moved to the other side

Apart from its simplicity the method presented has all the usual advantages of Goldmann perimetry e g exact adjustment of the brightness of targets and sphere variation in size and brightness of the targets control of fixa tion through the telescope

The different techniques are illustrated by two selected clinical cases

Case 1

A woman aged 54 blind in the left eye after uveitis At the age of 46 she complained of headache Roentgenograms of the skull showed an expanded sella turcica The right visual field appeared normal in kinetic perimetry until August 1974 when partial temporal hemianopia was recorded Roentgeno grams showed further enlargement of the sella and pneumoencephalographic findings suggested an empty sella syndrome

Colour saturation test with red targets (V/4) symmetrically projected on either side of the vertical meridian the patient reported desaturation of colour in both temporal quadrants the right target appearing yellow and less bright than the left one which was perceived as bright red (Fig 3)

Case 2

A man aged 56 was admitted to hospital with a left hemiparesis which had appeared suddenly A right carotid angiogram showed thrombosis of the inter

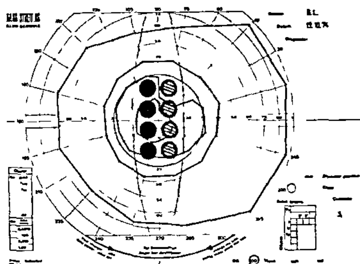


Fig 3

Case 1 woman aged 54 Empty sella syndrome Kinetic perimetry partial temporal hemianopia Colour saturation test desaturation of red in both temporal quadrants

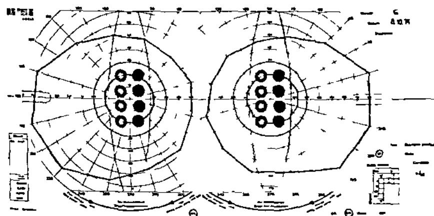


Fig 4

Case 9 man aged 56 Vascular lesion of the right hemisphere Kinetic perimetry normal fields Double point test left inattention hemianopia

nal carotid artery and signs of cerebral embolism The patient did not co operate well but presumably had a complete left homonymous hemianopia When examined 1 month later he had normal fundi visual acuity 1 0 bi laterally and normal visual fields with kinetic quantitative Goldmann peri metry When tested with the double point technique (white II/4) he had a left inattention hemianopia (Fig 4) With simultaneous and symmetrical pro jection of white targets on either side of the vertical meridian the patient only perceived the targets in the right hand field of either eye though he could see the targets to the left in single projection

Acknowledgement

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EFFECTS OF ARTIFICIAL INTRAOCULAR PRESSURE ELEVATION ON THE CORNEAL ENDOTHELIUM IN THE VERVET MONKEY
(Cercopithecus ethiops)

BY

BJÖRN SVEDBERGH

Both eyes of anesthetized vervet monkeys were perfused with mock aqueous humor for 3–7 hours By adjusting the height of a reservoir connected to the anterior chamber of each eye the intraocular pressure in one eye was maintained at 33–44 mmHg and in the other eye it was a few mmHg above the spontaneous level (12–15 mmHg) Morphologically the control eyes appeared normal whereas pronounced changes were observed in the high pressure eyes Thus the corneal endothelium showed an uneven surface towards the anterior chamber with vacuolization blebbing and disruption of the cytoplasm Pycnosis excaryocytosis and even loss of whole endothelial cells were observed as well The morphological changes were most pronounced in the peripheral part of the cornea and furthermore differed among neighbouring cells During the healing process one could observe mitosis amitosis and cell surface increase

Key words corneal endothelium – intraocular pressure – glaucoma – regeneration – autoradiography – transmission electron microscopy – scanning electron microscopy

Up until a century ago it was generally believed that the aqueous humor left the eye by passage via large pores straight through the cornea (Steno 1664) This belief was supported by the drop test i e drops of aqueous humor

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appeared on the corneal surface when an enucleated eye was squeezed (Leewenhoek 1684) However the classical works by Schwalbe (1870) and Leber (1873) clearly demonstrated that the aqueous humor left the eye mainly by passing through the trabecular meshwork Schlemm s canal and "clear vessels" – well fitting the earlier and later descriptions of aqueous veins (Knutsson & Sears 1973) – into the venous circulation Leber also confirmed that the drop test was a postmortal phenomenon and furthermore demonstrated that the corneal endothelium was the most important barrier to aqueous flow through the cornea His experiments showed that corneal opacities occurred at normal intraocular pressure (IOP) after mechanical injury to the endothelium a result confirmed by many later investigators and clinical experience (Donn 1966) On the other hand IOP levels around 200 mmHg were necessary in general to achieve opacities in the normal cornea However the physiological conditions of his experiments may be questioned (Ridley 1930)

More recent investigations dealing with the relationship between IOP corneal hydration and transparency have been reviewed by Ytteborg & Dohlman (1965a) Ehlers (1966b) and Maurice (1969) The former authors also performed experiments indicating that corneal epithelial oedema appeared when the corneal interstitial fluid pressure became positive (Ytteborg & Dohlman 1965b see also Donn 1966 and Mishima & Hedbys 1968) In experiments similar to those in the present report the IOP in the living rabbit was kept at 70 mmHg with stromal opacity appearing after 1 1/2 hours and *epithelial oedema after 5* hours This result was thought to be due to the direct effects of IOP on the normally functioning endothelium or more likely to damage to the endothelial cells They however presented no morphological evidence for such endothelial damage Such evidence was given by Leber (1873) describing localized endothelial defects corresponding to spots of corneal opacities in a rabbit at 110 mmHg duration not stated No other reports of morphological appearances at experimentally high IOP have been found until the recent preliminary report by Maurice & Hoefle (1973) They observed no change from normal appearance in transmission electron microscopy (TEM) when elevating the IOP to 60–80 mmHg *in vitro* duration not stated

In glaucoma Irvine (1956) in a study on 47 patients described flattening and attenuation of the endothelial cells The number of cells per unit area was usually decreased and in some cases the endothelium was totally absent Using scanning electron microscopy (SEM) Hervouet et al (1972 1973) observed expulsion of the nucleus and disappearance of the endothelium in large areas of the cornea Also using SEM Renard & Galle (1973) and Renard & Pouliquen (1974) studied two eyes with acute glaucoma and corneal oedema and noticed flattening of the cells with displacement of the nucleus towards the periphery

as well as disappearance of the intercellular pores and rarefication of the intercellular bridges

Thus high IOP may cause decompensation of the corneal endothelium i e it permits excess fluid to enter the corneal stroma from the anterior chamber (Donn 1966) Whether this decompensation is due to the high IOP per se or to a secondary damage to the endothelial cells is not evident from the reports above The purpose of the present study was to examine the effects of a moderate rise of IOP lasting some hours on the ultrastructure of the corneal endothelium and also to examine a possible repair process Preliminary reports as well as a separate study on the outflow facility and the ultrastructure of the chamber angle in the same eyes have been presented earlier (Svedbergh 1974a b c)

Material and Methods

Ten adult vervet monkeys (*Cercopithecus ethiops*) of both sexes weighing 2 15–5 65 kg were investigated No signs of disease in the anterior segment were observed by slit lamp examination

The complete *experimental and histological procedures* have been described earlier (Bill & Svedbergh 1972 Svedbergh 1974c) and are summarized as follows The experimental setup is shown in Fig 1 Three needles were shot into the anterior chamber of

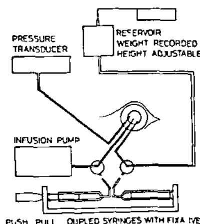


Fig 1

Schematic presentation of the experimental setup The anterior chamber of each eye was connected by three cannulae to a pressure transducer a reservoir and an infusion pump After the period of high intraocular pressure the reservoir and the infusion pump were disconnected and the push pull coupled syringes connected for fixation

each eye of the anesthetized animal One needle connected the eye to a pressure transducer continuously recording the IOP Another needle connected the eye to an infusion pump and the last needle connected it to a reservoir Most of the cornea was covered by the upper lid and wetting with saline was rarely necessary During the experiment the IOP was immediately elevated to 33–44 mmHg in one eye (high pressure eye) and to a few mmHg above the spontaneous level (1?–15 mmHg) in the other eye (control eye) by regulating the height of the reservoirs An equal flow through the anterior chamber of both eyes was accomplished by aid of the infusion pumps the rate for both eyes being adjusted similarily in order to balance the progressively increasing outflow from the high pressure eye (Svedbergh 1974c) The perfusion fluid was mock aqueous humor pH 7 4 (Barany 1964) After 3–7 hours elevation of the IOP the animals were killed by opening the heart and fixation immediately started by rapid perfusion (0 5–1 ml/min) of the anterior chamber with glutaraldehyde from the push pull coupled syringes The fixative perfusion lasted 10–15 min care being taken to maintain the IOP at the initial spontaneous level Three animals were killed and the eyes fixed by perfusion 1 3 and 10 days after the IOP elevation experiment in order to study the healing process After the perfusion of fixative the anterior segment was dissected in glutaraldehyde and postfixed in osmium tetroxide For scanning electron microscopy (SEM) different dehydration procedures were used – air drying from ether or ethanol freeze drying from amylacetate or 70 % ethanol – before final dissection For transmission electron microscopy (TEM) the embedding was done in Epon 812 via propylene oxide

Autoradiographical procedure 24 hours after the IOP elevation experiment 0 1 ml tritiated thymidine (Thymidine 6 ^{3}H specific activity 21 curies/mmol radioactive conc 0 5 mCi/ml Amersham) in mock aqueous humor was perfused by the push pull coupled syringes into the anterior chamber of both eyes in one animal After ? hours the animal was killed by opening the heart and fixation immediately started by perfusion with the push pull coupled syringes Further processing was performed basically according to Mills & Donn (1960)

Results

During the IOP elevation no distinct corneal opacity could be observed by slit lamp examination (magnification × 2) except in the neighbourhood of the needle holes However with a magnification × 10 – × 25 a patchy haze in the anterior stroma of the high pressure eyes could be observed after about 2 hours followed shortly by epithelial bedewing i e small clear vesicles sub and intra epithelially (also observed by microscopy) No large changes in corneal thickness were noted by slit lamp examination and light microscopy Occasionally a slight patchy stromal haze could also be observed in the control eyes but never any epithelial bedewing When selecting areas for studying morphological changes due to the IOP elevation care was taken to avoid areas adjacent to the needle holes and the dissection borders The endothelium of the control eyes had an appearance in accordance with earlier studies on normal morphology with TEM

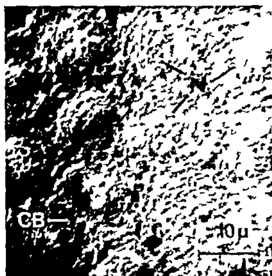


Fig 2

Slight damage by the IOP elevation The cell surface becomes uneven the nuclei (N) are readily observed whereas the cell borders (CB) are difficult to identify Compare with the normal appearance in Fig 8 SEM preparation air dried from ether

(Iwamoto & Smelser 1965 Hodson 1968 Hogan et al 1971 Wulle 1972) and SEM (Svedbergh & Bill 1972 Renard & Galle 1973 Doughman et al 1974)

In the high pressure eyes fixed immediately after the IOP elevation experiment a wide spectrum of morphological lesions occurred in one and the same eye the peripheral part being more susceptible than the central part Even neighbouring cells showed different degrees of cellular damage The slightest changes consisted of an increasing unevenness of the cell surface towards the anterior chamber with less distinct cell borders (Fig 2) Cilia were observed less frequently than in the control eyes The cells were often flattened to a varying degree occasionally as thin as 0 5 μ (normal height about 5 μ) They further showed differing electron optical cytoplasmatic densities between the neighbouring cells indicating fluid accumulation or cytoplasmatic extraction in the lighter cells or possibly differences in metabolic activity The electron optical density also varied within the individual cell mostly with the lowest densities adjacent to Descemet s membrane Vacuolization occurred both intra and intercellularly Swelling of the mitochondria and the endoplasmatic reticulum was observed as well as discontinuities of the terminal web The intercellular space and the interface towards the De cemet s membrane appeared partly distended but still with intact junctional structures Also the perinuclear space was slightly

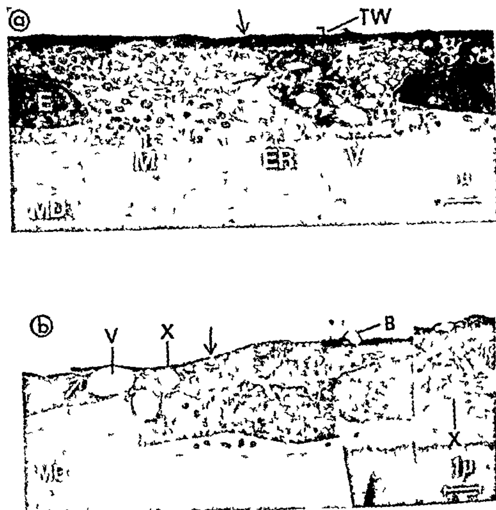


Fig 8

Slight and ꞏ ꞏ re damage by the IOP elevation TEM a) The endothelial cells (E) are flattened ꞏ ga n ꞏ Descemet s membrane (MD) Neighbouring cells show different electron optical ꞏ t ꞏ asmatic density Vacuoles (V) swollen mitochondria (M) and endoplasmatic ꞏ ticulum (ER) are observed as well as slight distension of the perinuclear space the intercellular space and the interfase towards Descemet s membrane Intercellular junctions (arrows) are intact The terminal web (TW) is continuous b) The endothelial ꞏ ells demonstrate differing electron optical densities and heights A vacuole (V) or a dilatation is seen in the intercellular space and the apical junction appears split (white arrow) The terminal web is discontinuous with vacuoles or vesicles (black arrow) opening into the anterior chamber The subnuclear region towards Descemet s membrane (MD) shows very low electron optical density A bleb (B) is ruptured The double membraned structure (X) is probably an extension of a neighbouring cell or possibly a naked nerve axon

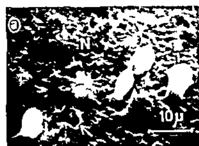




Fig 4

Severe damage by the IOP elevation, a) The cytoplasmatic membranes have disrupted at many places (arrow) the nuclear bulges (N) are distinct and blebs (B) containing cytoplasm or nuclei are prominent. SEM preparation air dried from ethanol b) The blebs bud off or burst emptying their content thus leaving craters behind (CR) Even whole cells may be lost exposing Descemet s membrane (MD) N denotes nuclear bulges SEM preparation air-dried from ethanol

distended (Fig 3a b) More severe changes consisted of progressive loss of intracellular organization loss of cilia and occasional disruption of the apical cell junction (Fig 3b) The nuclei rounded up Balloon like protrusions so called blebs were observed budding off or bursting thereby emptying their content which could be either cytoplasmatic material or the nucleus (excaryocytosis) These budding or bursting blebs left gaping craters behind Even whole cells could be lost leaving only fragmentary remains on the Descemet s membrane (Figs 3b 4a b)

Preliminary observations on the three eyes fixed 1 3 and 10 days after the IOP elevation experiment indicated that the repair process was quite efficient since neither stromal patchy opacities nor epithelial bedewing were observed after 3 and 10 days However ultrastructural signs of the healing process were evident Thus some cells had a very large surface area with a rounded instead of a polygonal form occasionally even with a waist The nucleus had rounded up or was bilobed or even divided Spoke like folds running from the nucleus to the periphery of the cells were conspicious In all the appearance of these cells strongly suggested different stages of cell division (Figs 5a b 6 7) The occurrence of cell division was confirmed by the autoradiographical study with tritiated thymidine It demonstrated many labelled nuclei in the peripheral part of the high pressure eye whereas no labelling could be observed in the control eyes (Fig 5b) The neighbours to such a dividing cell demonstrated dorsal ripples on their adjacent cytoplasmatic membrane possibly an expression for locomotion of these cells or a pushing by the dividing cell (Fig 5a 6 7) Amitosis



Fig 7

Healing process The central structure is probably a very large irregular cell with two nuclei in telophase The nuclei are not separated by any observable cell border but instead there is a waist on the cell suggesting a cleavage furrow or contractile ring (arrows) SEM 3 days after the IOP elevation air dried from ethanol

also took place as suggested by occasional cells with more than one nucleus and duplicated cilia (Fig 8) However no giant multinucleated cells were observed *Defects in the endothelial cell layer* also seemed *to be covered simply* by cell enlargement as indicated by the existence of occasional very large mononuclear polygonal cells

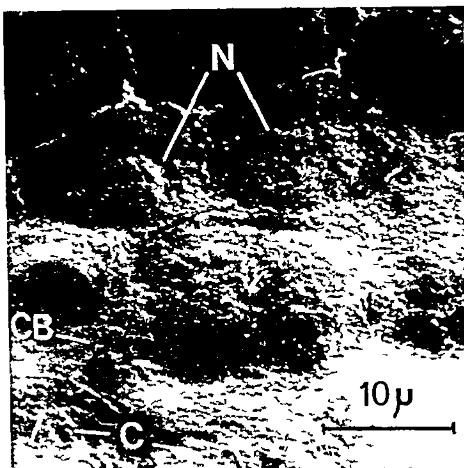


Fig 8

Healing process The nucl ı (N) the cell borders (CB) and cilia (C) are ol served In the middle there is a large endothelial cell with duplicated nuclei and cilia demonstrating amitosis SEM 10 days after the IOP elevation experiments air dried from ethanol

Discussion

In experiments with perfusion of the anterior chamber the composition of the perfusion fluid may influence the ultrastructural appearance (Kaye et al 1973) especially at a high perfusion rate (McCarey et al 19,3) In the present control eyes no adverse effects coul. be at ribu ed to the perfusion fluid the high perfusion rate or the corr al n edles (except adjacent to the needle holes) It seems

likely therefore that the cellular damage in the high pressure eyes was mainly caused by the high IOP per se

Normally the corneal endothelium acts as a barrier to excessive aqueous humor flow into the cornea When the endothelium decompensates excess aqueous humor is allowed to pass into the corneal stroma with resulting stromal opacities – with or without considerable stromal swelling (Ytteborg & Dohlman 1965a Ehlers 1966a Goldmann & Kuwabara 1968 Hodson 1974) – and epithelial bedewing (Donn 1966) At high IOP the endothelium may decompensate There is however uncertainty as to whether this decompensation is due to cell damage and thus has a morphological counterpart (Leber 1873 Ytteborg & Dohlman 1965a b Donn 1966 Maurice & Hoefle 1973) In the present report the observed morphological changes – unevenness of the cell surface vacuolization loosening of the cell junctions blebbing disruption of the plasma membrane excaryocytosis loss of the whole cells – clearly indicate damage to the corneal endothelium by a moderate experimental IOP elevation *in vivo* of a duration of more than 3 hours Gross endothelial decompensation as reflected by the epithelial bedewing observed after about 2 hours would readily be explained as secondary to the endothelial damage The results suggest that the duration of the IOP elevation is an important factor to consider when judging effects on morphology Slight endothelial decompensation if reflected by discrete stromal patchy opacities alone was here not accompanied by any detectable morphological changes judging from the normal appearance of the control eyes with such opacities

The following tentative explanation is proposed as to how the elevated IOP affects the corneal endothelium The fluid passage across the endothelium is nowadays believed to be regulated by a pump leak system (Maurice 1969 Maurice et al 1973 Fischbarg 1973 Bito et al 1973 Fischbarg & Lim 1974 Hodson 1974) i e there is a passive leakage of fluid from the anterior chamber through the apical junctions and the intercellular spaces towards the stroma This flow is counteracted by an active fluid pump in the endothelial cells which transports fluid from the cell into the anterior chamber However the basic properties of both the cell junction (Diamond 1974) and the active pump (Wilbrandt 1975) have not been clarified Pure tonicity forces may also be involved in the fluid passage across the endothelium (von Bahr 1956) When the IOP is elevated there is an increased leakage through the intercellular spaces proportional to the level of the IOP This leakage is counteracted by the fluid pump up to a certain IOP level 60–80 mmHg in rabbit *in vitro* duration not stated (Maurice & Hoefle 1973) A second effect of the elevated IOP is an increased compression of the cells against Descemet s membrane (Kaye et al 1973) as well as an increased overall compression of the cells A third effect is a distension of the whole bulb

causing a lateral stretching of the endothelial cells although this effect is expected to be small and transient (Ridley 1930 McEwen 1967 1972) The two las effects i e compression and lateral stretching tend to flatten the cells and dilate the intercellular spaces with subsequent strain on the cytoskeleton in general As time goes by this strain appears to break down the structural barriers to fluid passage as indicated by the observed unevenness of the cell surface the discontinuities of the terminal web the partly dilated intercellular spaces and occasional splitting of the apical cell junctions Also the phenomenon of bleb bin is consistent with a weak or absent cytoskeleton (Harris 1973) and known to occur not only as a result of bad fixation but also at malfunction of the cells and at initial stages of regeneration (Price 1967 Trinkaus 1973 Van Horn & Schultz 1974) This structural damage and/or the compression itself impair the function of the cell as indicated by the vacuolization the swollen cell organelles the cytoplasmatic extraction and the progressive loss of intracellular organization The nucleus is not so readily flattened but rounds up instead This seems to result in rupture or blebbing of the overlying plasma membrane and expulsion of the nucleus At this stage there remains very little structural basis for the existence of a hydraulic resistance and a tonicity gradient across the endothelium The aqueous humor now passes easily into and between the cells and accumulates in the region towards Descemet's membrane with the eventual separation of whole damaged cells Fluid also passes further forwards causing stromal opacities eventual stromal swelling and epithelial bedewing The flow through the cornea at high IOP may be considerable Experimental measurements (Ridley 1930) as well as theoretical considerations (Mishima & Hedbys 1967 Yasuda & Stone Jr 1967 Fischbarg 1973) suggest a flow range of about 1-9 μl/min

Regarding regeneration the present findings mitosis amitosis cell surface increase cell movement – are basically in accordance with earlier studies on the subject (Eberth 1876 Schottlander 1888 Peters 1889 Donn 1966 [illegible] 1971 Bronstein 1973 Sanchez & Polack 1974 Doughman et al 1974) [illegible] disappearance of the stromal patchy opacities and the epithelial bedewing [illegible] days is probably due to the fact that small areas of endothelial [illegible] faster than large ones (Leber 1873 Chi et al 1960 Donn 1966 [illegible]) The cell enlargement observed here is known to precede or even [illegible] division (Donachie 1968 Wessels et al 1973) The cytoplasmic [illegible] folds probably correspond to the tenous extensions (Boyde et al 1972) or the retraction fibers (Middleton 1973) whereas the appearance of a [illegible] cell may correspond to the contractile ring (Schroeder 1970) These features together with the nuclear rounding up strongly suggest mitosis (Boyde et al 1972) whereas the occasional occurrence of binuclear cells in interphase and [illegible]

amitosis However multinucleated giant cells (Schottlander 1888 Laure et al 1971) were not found in the present eyes The dorsal ridges on the neighbouring cell edges are possibly effects of pushing by the dividing cell and/or related to the phenomenon of "ruffling" in cell movement (Abercrombie et al 1970 Boyde et al 1972 Sanchez & Polack 1974)

In glaucoma morphological changes similar to those of the present report have been observed Thus flattening and attenuation of the endothelium (Irvine 1956 Renard et al 1973 1974) excaryocytosis and loss of whole cells have been described (Irvine 1956 Hervouet et al 1972 Hervouet & Lrtus 1973) The intercellular pores described by Renard & Galle (1973) and Renard & Pouliquen (1974) are probably artefacts of the drying procedure and/or electron beam bombardment (Svedbergh & Bill 1972) Clinically it is well known that high IOP may occur without corneal opacity and epithelial oedema in chronic glaucoma (Irvine 1956 Ytteborg & Dohlman 1965b) In acute glaucoma the opacity and oedema is a far more consistent sign (Ridley 1930 Jones 1959 Boruchoff 1968 Goldman & Kuwabara 1968) It seems likely that in acute glaucoma there is rapid and marked damage of the endothelial cells – as in the present experiments – whereas in chronic glaucoma the IOP elevation and the damage to the endothelial cells are more gradual making continuous healing possible Furthermore in acute glaucoma – where the normal outflow apparatus is blocked – the corneal damage permits a flow through the cornea (see above) which might operate as an emergency exit for aqueous humor arresting further IOP elevation and maintaining some circulation and nutrition The corneal damage might also be the prerequisite for the development of corneal aqueous veins in longstanding glaucoma (see Ascher 1961)

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CONTACT PROBE FOR CORNEAL TEMPERATURE MEASUREMENTS

BY

I HØRVEN and C T LARSEN

A thermistor probe for corneal temperature measurements is presented The temperature sensing cartridge is similar in shape to an electronic tono meter and the procedure of corneal temperature registration is performed just as easily and in a similar manner as for tonography

Key words corneal temperature – eye temperature – ocular temperature – temperature – thermistor

Ocular temperature registrations may be performed either by direct or indirect means All indirect methods measure the infra red radiation emitted by the eye and deduce the average temperature over the emitting area from the Stefan Boltzman law with an assumed average emissivity of the corneal surface Instru ments of this sort may be difficult to calibrate and have a high initial cost They have the advantage that registrations are performed on the undisturbed eye without the need for topical anesthesia Such a radio metric instrument was introduced by Zeiss (1930) and the technique was later improved and used by Mapstone (1968a b) and Rysa & Sarvaranta (1974)

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The direct approach for ocular temperature registration has been performed either by the use of specially constructed mercury thermometers (Dohnberg 1876 Galezowski 1877 Hertel 1900 and others) or by thermo electrical instruments Von Michel (1886) introduced such an instrument for ocular temperature measurements in animals while Silex (1893) Giese (1894) Muto (1931) Braendstrup (1952) Holmberg (1952) Matthaus (1961) and others also performed registrations in human beings Schwartz & Feller (1962) Schwartz (1965) and Freeman & Fatt (1973) studied temperature gradients and environmental influences upon the ocular temperature in rabbits While the early authors recorded the conjunctival temperature at the fornix inferior Muto (1931) was the first ever to measure the corneal temperature by the direct approach

When a probe sensor comes in contact with the cornea there is a transient change in the corneal temperature directly below the probe tip due to the difference in the initial temperatures of the probe and the cornea To obtain good thermal contact the sensor in the probe must exert some pressure on the cornea. To some extent the pressure of the probe alters the rate of heat transfer from the cornea because the probe conducts heat from the corneal surface and prevents normal radiation and convection from that area of the cornea which it covers The heat conducted to the probe is then radiated and convected from its surface a process called fin effect in engineering heat transfer (Kreith 1965)

Recently Guadagni et al (1972) presented a thermistor contact probe for skin temperature measurements The thermistor itself is 0 0125 inch in diameter which is the smallest glass coated thermistor commercially available In the steady state the probe acts as a fin on the skin It does this because it conducts heat away from the surface of the skin and radiates and convects this heat from its own surface which has a larger heat transfer area than the area of the skin which the probe tip covers However by constructing the outer probe tip socket from aluminium which has a small surface emissivity the rate of heat transfer by radiation from the probe is less than it was from the area of skin which the probe tip covers Thus with this probe design the total rate of heat transfer from the probe is only slightly greater than it would be from the undisturbed skin and the fin effect is small The calibration accuracy of the thermistor is better than 0 01 C and the probe reproductibility is excellent (Guadagni et al 1972) In the present study such a thermistor probe was modified and used for corneal temperature registrations

Instrumentation

The thermistor used is ITT type U 23 US (NTC resistor ca 1 2 kΩ at 36 C) Fig 1 shows the electronic circuit used to convert the variations in resistance to

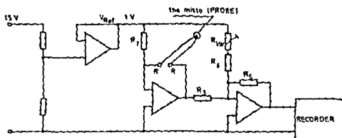


Fig 1
Schematic view of the electronic circuit of the corneal thermometer

variations in a voltage V_{out} which can be recorded or measured on a voltmeter In the present study a Brush Mark 220 recorder was used

The thermistor R_0 ΔR is used as the feed back resistor in an operational amplifier to ensure a constant current in the thermistor This constant current is given by $\frac{V_{REF}}{R_1}$ and is approximately 30 μA Variations in V_1 and V_{out} are thus proportional to ΔR V_{out} is given by the equation

$$V_{out} = -\frac{R_3}{R_1 \quad R_2} \quad \Delta R \quad V_{REF} + V_0$$

where V is a voltage adjustable by R_{VAR}

The circuit is designed to give an output signal of 1V for $\Delta R = 300\ \Omega$ Calibration of the instrument should be accomplished by measuring two defined temperatures with the probe By measuring the difference in V_{out} the sensitivity $\frac{\Delta V_{out}}{\Delta T}$ can be calculated

The thermistor probe consists of a temperature sensing cartridge supplied with a sensing tip as shown in Fig 2 The weight is 5 g and the contact area of the sensing tip is about 7 mm which gives a contact pressure between the probe and the corneal epithelium of ca 0 7 g mm ² This equals 1 0 lb in and is within the range of 0 6– 1 0 lb in recommended by Guadagni et al (1972) During registration the thermistor probe rests on the corneal surface by its own weight similar to an electronic tonometer Thus the contact pressure is easily kept within the required range which is necessary in order to get stable correct results A contact pressure which is too low will give poor thermal contact between probe and cornea with unstable temperature readings as a result whilst a contact pressure which is too high will indent the cornea towards the warmer

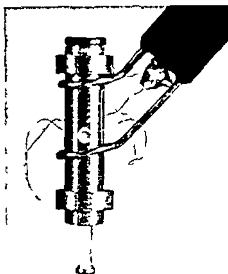


Fig 2
The sensing cartridge of the corneal thermometer

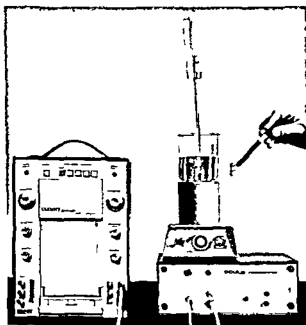


Fig 3
The equipment used for clinical measurements of the corneal temperature

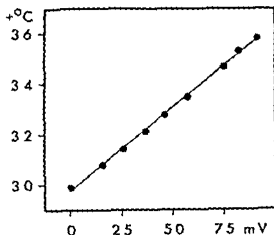


Fig 4

A fairly linear relation exists between temperature in °C and output in mV

structures of the inner eye (Matthaus 1961) thus giving too high temperature results As shown by Guadagni et al (1972) at pressures above 1 0 lb in the steady state probe skin temperatures were found to be significantly pressure dependent Too high a contact pressure should therefore be avoided

Calibration

A water bath supplied with a magnet rotor and a thermometer which permits readings to 0 10 C is used for calibration and for reference temperatures The equipment is shown in Fig 3 By placing the thermistor probe on the water readings could be made at various temperature levels As seen in Fig 4 the output was fairly linear within the tested temperature range of 30–36°C For the thermistor probe used in the present study the output was 13 6 mV per °C With a sensitivity setting of 5 mV per paper division of the recorder this gives 2 7 divisions per C The curve tracings can easily be read with an accuracy of ½ division which by the 5 mV per division sensitivity setting equals about 0 2 C as seen in Fig 5 If a sensitivity setting of 2 mV per paper division is used the output is 6 8 divisions per °C In this case temperature differences less than 0 10 C can easily be detected in the recordings (Fig 5)

As seen from Fig 5 the corneal temperature is recorded in two sequences First the right and left corneal temperatures are recorded allowing the probe to

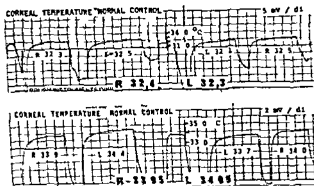


Fig 5

Corneal temperature measurements in two normal subjects Sensitivity settings 5 mV per paper division (above) and 2 mV per paper division (below)

rest for 15–20 sec on each eye Then a reference temperature is recorded As seen from the Figure the temperature reading on water is almost instantaneous Finally the corneal temperatures are recorded in reversed order The average values of the right side and the left side are calculated By this procedure of corneal temperature recording the possible influence of a reflectory increase in temperature on the second eye is minimized This will be more fully discussed in a following paper (Hørven 1975)

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CORNEAL TEMPERATURE IN NORMAL SUBJECTS AND ARTERIAL OCCLUSIVE DISEASE

BY

IVAR HORVEN

A direct thermo electrical method of corneal temperature recording is presented The corneal temperature averaged 33 C in normal subjects with no side difference and no difference between the sexes The various parameters which may influence the corneal temperature are discussed The technique was applied to groups of patients suffering from either polymyalgia rheumatica central retinal artery embolism or temporal arteritis In the latter group of patients a marked drop in corneal temperature was demonstrated in the affected or most affected eye Corneal temperature measurements are therefore presented as an easy and effective diagnostic approach in temporal arteritis

Key words age – arterial occlusive disease – arteritis temporalis – central retinal artery embolism – corneal emperature – ocular temperature – oral temperature – temperature – thermistor probe

In 1900 Hertel observed a 0 78 C drop in ocular temperature in rabbits following a 3 min compression of the ipsilateral common carotid artery thus demonstrating that the temperature at least in the anterior part of the eye is dependent upon the ocular blood supply Colle et al (1931) found a 2–4 C decrease in anterior chamber temperature following ipsilateral internal carotid artery occlusion in cats and dogs When they applied compression over the abdominal aorta

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thus forcing more blood to the animal s head a corresponding increase in ocular temperature was noted In four patients with monolateral carotid artery stenosis Mapstone (1968a) demonstrated lower corneal temperatures on the affected side The decrease averaged 1 0°C (0 8–1 2)

These studies indicate that accurate recording of corneal temperature may be used as a valuable clinical approach to the diagnosis of the arterial occlusive diseases affecting the ocular blood supply In order to proclaim pathological results a proper knowledge of the corneal temperature pattern in normal subjects is mandatory A control group is therefore included in the present study

Instrumentation

The corneal temperature was recorded by a specially constructed thermistor probe (Horven & Larsen 1975) and a Brush Mark 220 recorder The equipment yielded an output of 13 6 mV per °C With a recorder sensitivity setting of either 5 or 2 mV per paper division the temperature tracings could be read with an accuracy of 0 2°C or less than 0 1°C respectively (Horven & Larsen 1975)

Methods

Allowing proper time to equilibrate in the room in which the temperature was kept bet en 21 5–24 5°C the subject was placed in the supine position and given t o drops of oxibuprocain 0 4 % in each eye A reference temperature was recorded by placing the thermistor probe in water with known temperature Afterwards the water remaining on the probe tip was removed The subject was asked to blink and a recording was performed first on the central part

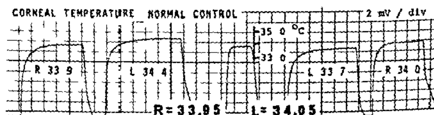


Fig 1
Corneal temperature measurement in a normal subject Sensitivity setting 2 mV per paper division

of the right cornea then on the left Another reference temperature was recorded the probe tip cleaned and following blinking the corneal temperature measurements were performed in reversed order Then subsequent reference temperatures were recorded and the probe tip cleaned with ethanol The oral temperature was measured by the use of a mercury thermometer

Material

Control material Forty normal subjects were examined eight in each of the 10-year age groups between 20 and 70 years There were 22 women and 18 men

Polymyalgia rheumatica Nine consecutive patients with polymyalgia elevated erythrocyte sedimentation rate but without ocular symptoms were included The average age was 66/ years (52–86)

Central retinal artery (CRA) embolism Eight consecutive patients with a fresh monolateral retinal artery embolism were measured Two of the patients had a branch occlusion the others had a complete CRA occlusion The average age was 65 3 years (54–75)

Temporal arteritis Seven consecutive patients with a biopsy proven temporal arteritis were included four had bilateral and three monolateral eye involvement Two of the patients were examined 4 years after steroid treatment had started at a time when the ocular blood supply had returned to fairly normal levels as judged by the dynamic tonometry results (Horven 1973) The corneal indentation pulse amplitudes which initially were low had at this time improved to almost a normal level The other five patients had an active disease The average age was 74 5 years (68–78)

Results

Control material All of the normal subjects were examined with a recorder sensitivity setting of 2 mV per paper division as seen in Fig 1 The average corneal temperature on the right side was 33 74 C (32 25–34 9) and on the left side 33 67 C (32 0–34 9) The difference in average temperature between the two eyes was 0 07 C which was not statistically significant as judged by the statistical method of paired comparison ($t = 0.463$) The standard deviation (s d) of the side difference was 0 23 C indicating that a side difference above 2 58 0 23 C = 0 59 C will normally be found in less than one out of a hundred

subjects A repeated side difference between the two eyes of 0 6°C or more is therefore accepted as pathological

The average corneal temperature was 33 67°C in women and 33 83°C in men the difference was not statistically significant (Student s t test $t = 0\ 878$)

As demonstrated by Braendstrup (1952) measuring corneal temperature on one eye initiates an increase in the recorded corneal temperature of the other eye She suggested the term "reflectory increase for this temperature rise although its origin is obscure A similar temperature rise was found in the present study as seen in Table I and Fig 1 In the first sequence the average corneal temperature of the second left eye was 0 295°C higher than that of the first recorded eye During the time required for reference temperature recording the recorded temperature of the left eye dropped to the value first recorded in the right eye However an increase occurred in the right eye too when this eye was recorded last in the second sequence This increase averaged 0 435°C Therefore in order to minimize the influence of this temperature rise the procedure suggested in the present study to record the average values of two sequences of which the second is performed in reversed order should be followed

In order to test the possible relationship between corneal temperature age and environmental factors the results from the 40 right eyes were used as seen in Fig 2

The *oral temperature* averaged 36 67°C (36 1–37 3) and the *room temperature* 23 13 C (21 5–24 5) A positive correlation was demonstrated both between corneal and oral temperature and between corneal and room temperature as seen in Fig 2

Age A negative correlation was found between age and corneal temperature significant at the 0 1 % level as seen in Fig 2 Table II offers the results from the various 10-year periods The decrease in corneal temperature with age is

Table I

Corneal temperature in C average values of 40 normal subjects An increase in temperature is noted in the last recorded eye in each sequence

First sequence			Second sequence		
Right eye	Left eye	Left right eye	Left eye	Right eye	Right left eye
33 540	33 835	0 295	33 510	33 945	0 435

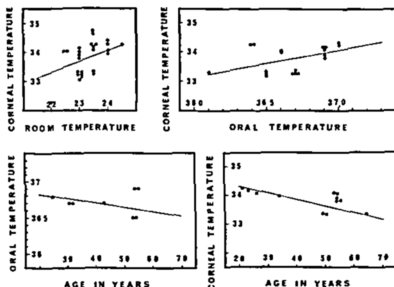


Fig 2

Positive correlation between cornea (y) and room (x) temperature $y = 0.372x + 25\ 13$ ($r = 0\ 431$ $P < 0\ 01$) Positive correlation between corneal (y) and oral (x) temperature $y = 0\ 937x - 0\ 45$ ($r = 0\ 399$ $P < 0\ 02$) Negative correlation between oral temperature (y) and age (x) $y = -0\ 0061x + 36\ 94$ ($r = -0.324$ $P < 0\ 05$) Negative correlation between corneal temperature (y) and age (x) $y = -0\ 023x + 34\ 74$ ($r = -0\ 523$ $P < 0\ 001$)

Table II

Temperature in C

Age	N	Room		Mouth		Right cornea	
		C	SD	C	SD	C	SD
20–29	8	23 13	0 [illegible]9	[illegible] 74	0 20	34 09	0 46
30–39	8	23.31	0 4[illegible]	3[illegible] [illegible]0	0.27	34 04	0.5[illegible]
40–49	8	23 19	0 70	36 [illegible]3	0 17	33 73	0 [illegible]2
50–59	8	23 0[illegible]	0 [illegible]	[illegible] 83	0 [illegible]7	3 80	0.3[illegible]
60–69	8	2[illegible] 94	0 62	3[illegible] 43	0 29	3 03	0.52
20–69	40	23 13	0 [illegible]	[illegible]6 [illegible]7	0 27	33 74	0 [illegible]2

Table III
Corneal temperature in °C

N	Polymyalgia rheumatica			CRA – embolism			Temporal arteritis		
	Right eye	Left eye	Diff	Affected eye	Normal eye	Diff	Most affected eye	Other eye	Diff
1	31.5	31.5	0	31.9	31.4	+0.4	30.7	32.4	−1.7
2	32.3	32.0	+0.3	32.4	32.2	+0.2	31.3	32.6	−1.3
3	32.4	32.4	0	34.1	33.5	+0.6	31.3	32.7	−1.4
4	32.4	32.0	+0.4	33.3	32.7	+0.6	31.6	33.0	−1.4
5	31.3	31.4	−0.1	32.2	32.2	0	32.2	32.9	−0.7
6	32.1	32.0	+0.1	32.5	32.6	−0.1	33.4	33.5	−0.1
7	32.9	33.0	−0.1	32.7	32.8	−0.1	33.3	33.0	+0.3
8	33.2	33.3	−0.1	34.4	34.1	+0.3			
9	34.0	33.6	+0.4						
Av	32.46	32.36	+0.1	32.94	32.69	+0.25	31.97	32.67	−0.9
t		1.414			2.376			3.154	
Significance		–			$P < 0.05$			$P < 0.02$	

* Statistical method of paired comparison

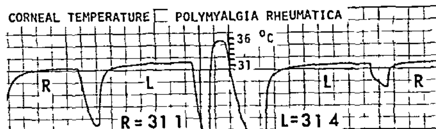


Fig 3
Corneal temperature recording in polymyalgia rheumatica Sensitivity setting 5 mV per paper division

most pronounced in subjects above 60 years of age When the eight subjects above 60 years of age were compared with the eight subjects between 20–29 years an average difference in corneal temperature of 1 0°C was found The Wilcoxon rank test for two samples gave the following ranks $N_1 = {}^{40}/_{8}$ $N = {}^{96}/_{8}$ indicating that a statistically significant difference exists between the two samples ($P < 0.01$)

A negative correlation was also found between age and *oral temperature* When the results from the eight oldest subjects were compared with the 20–29-year group an average difference of 0 31°C was found The Wilcoxon rank test for two samples demonstrated a statistically significant difference between the two samples ($N_1 = {}^{48.5}/_{8}$ $N = {}^{87.5}/_{8}$ $P < 0.05$)

Polymyalgia rheumatica The results are listed in Table III and a typical recording from a patient examined with a recorder sensitivity setting of 5 mV per paper division is presented in Fig 3 No side difference was found

Central retinal artery occlusion The results are listed in Table III and a typical recording is shown in Fig 4 below A small side difference was present Two of the patients showed an increase of 0 6 C on the affected side in the other six patients the side difference was within the normal range However on average the corneal temperature was 0 25 C higher on the affected side The difference was statistically significant at the 5 % level (Table III) As previously shown (Hørven 1973) no side difference is present in corneal indentation pulse amplitudes by CRA occlusion

Temporal arteritis The results are listed in Table III and a typical recording shown in Fig 4 In the five patients with active disease a marked side difference in corneal temperature was found with lowest values on the affected or most

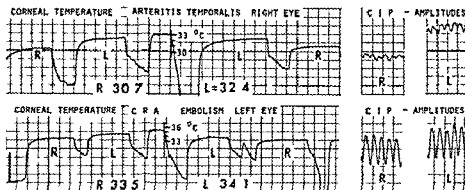


Fig 4

Corneal temperature decrease and corneal indentation pulse (C I P) amplitude reduction in temporal arteritis (above) Corneal temperature increase in central retinal artery embolism (below) Sensitivity setting 5 mV per paper division

affected eye The average difference was –1 3°C (–0 7 to –1 7) The two patients in which no side difference was found were examined in the inactive stage 4 years after steroid treatment had been started One patient was examined repeatedly The initial side difference of –1 7°C showed a minor decrease following steroid treatment being –1 65°C and –1 4°C after 1 and 8 months respectively Including all seven patients the side difference proved to be statistically significant at the 2 % level (Table III) Including only the five patients with active disease a *t* value of 7 912 was found ($P < 0\ 005$) The 31 42°C average value of the most affected eye in these five patients was significantly lower than the 33 08°C average value demonstrated in the eight normal subjects above 60 years of age (Wilcoxon rank test for two samples $N_1 = {}^{15\,5}/_5$ N = $/_8$ $P < 0\ 01$) All of the five patients with active disease showed a pathological side difference of 0 7°C or more (Table III)

Comment

Using a thermo electrical instrument for direct recording of the corneal temperature Muto (1931) found an average value of 30 2°C while Braendstrup (1952) and Matthaus (1961) gave average values of about 32 0 C When a radiometric indirect approach is used average values of 34 8 C are found (Mapstone 1968b Rysa & Sarvaranta 1974) However when an indirect method is used the radiation from the entire corneal surface is recorded The average

value should therefore be higher than that of the central part of the cornea, as the corneal temperature increases towards the limbal zone (Braendstrup 1952). In addition the indirect radiometric method also receives emission from the tear film which presumably is somewhat warmer than the corneal tissue. Accordingly the average value obtained by radiometric methods is probably somewhat higher than the actual temperature in the central part of the cornea. The thermistor probe used in the present study has a contact area of only 7 mm which rests directly upon the corneal tissue shielded from the tear film by the outer socket of aluminium (Horven & Larsen 1975). The temperature sensing cartridge is specially designed to minimize the fin effect of the thermistor so that the total rate of heat transfer from the probe is only slightly larger than it would be from the undisturbed cornea. The corneal temperature is therefore only to a negligible extent influenced by this procedure of recording. The average value of 33.7°C found in the present study should therefore give a fair estimate of the actual temperature of the central part of the normal human cornea. As mentioned earlier results obtained by the direct thermo electrical approach gave lower values. This may be explained by the fact that these authors used larger probes which presumably had a larger fin effect, convecting and radiating heat away from the cornea to be tested. In the present study the difference in temperature between mouth and cornea was about 3.0°C. Measuring the cul de sac conjunctival temperature, Howe (1913) found a difference of 0.3–0.4°C while Holmberg (1952) found the conjunctival temperature close to the limbal area to be on average 2.5°C (2.1–2.9) lower than that of the mouth. This agrees favourably with the above results, as Braendstrup (1952) has shown that the limbal temperature is about 0.5°C higher than that of the central part of the cornea.

It was previously shown that the temperature in camera anterior (von Michel 1886) and cornea (Braendstrup 1952, Mapstone 1968a) increases with lid closure. A certain rise may also be noted following rapid blinking and increased tearing (Mapstone 1968a). Relative humidity over a substantial range has a minimal effect upon corneal temperature (Schwartz 1965). Air movement, however, may change the corneal surface temperature in a most striking way (Braendstrup 1952, Freeman & Fatt 1973). Open windows and drafts should therefore be avoided during recording, otherwise a decrease in corneal temperature may occur. If the lids are retracted by a speculum, a decrease in corneal temperature is also observed (Braendstrup 1952, Mapstone 1968a).

The positive correlation between corneal and room temperature has also been found by a number of authors (Hertel 1900, Braendstrup 1952, Schwartz 1965, Mapstone 1968a, Freeman & Fatt 1973, Rysa & Sarvaranta 1974). The change in corneal temperature per degree room temperature was calculated to

0.145°C by Mapstone (1968a) to 0.23°C (in rabbits) by Schwartz (1965) and to 0.37°C in the present study Although small within the normal range of room temperature (18–25°C) this influence should not be neglected The positive correlation between body and corneal temperature has also been described previously (Braendstrup 1952 Matthaus 1961)

The negative correlation between corneal temperature and age was briefly mentioned by Braendstrup (1952) but has not been the subject for statistical evaluation before As mentioned the decrease in corneal temperature was most striking in subjects over 60 years of age The difference of 1.0°C as demonstrated between young and old subjects in the present study can only partly be explained by a difference in body temperature as the difference in oral temperature between the two groups was only 0.31°C No difference was present between the two groups as concerns room temperature and other parameters mentioned above which may alter the corneal temperature The low corneal temperature recorded in subjects over 60 years of age may therefore mainly be attributed to the aging process probably reflecting a certain age induced arteriosclerotic reduction in ocular blood supply Temperature is one of the fundamental parameters of tissue metabolism If the average body temperature of a man is lowered by 10°C the metabolic rate would be reduced by a factor of 2.5 (Hardy & Bard 1974) A drop in corneal temperature of 1.0 C may therefore decrease the metabolic rate by 10% The corneal temperature is maintained by heat transfer from the tear film from the vascularized limbal area and primarily from the aqueous humour A drop in corneal temperature reflects therefore most probably a similar although less pronounced decrease in lens temperature with a corresponding reduction in lens enzyme reactions and metabolic rate This may probably be sufficient to play a part in the pathogenesis of senile cataracts A decrease in corneal temperature will imply an increase in the temperature gradient within the eye provided that the body and posterior eye temperature are unchanged Physiologically such an increased temperature gradient may affect the transport of substances within the eye The temperature gradient force may have a significant role in maintaining intraocular pressure (Fremont Smith 1961) Therefore an increased temperatur gradient could possibly explain the minor increase in intraocular pressure observed with age (Leydhecker 1960 Armaly 1965) Bengtsson (1972) on the other hand believes this increase in intraocular pressure with age might be explained by a concomitant increase in systemic blood pressure

The present study also confirms that an increase in temperature occurs when the second eye is recorded In the series presented by Braendstrup (1952) an average increase of 0.34°C was found which corresponds very well to the values of 0.295°C and 0.435°C observed in the present study The mechanism behind

this increase in temperature is obscure The recording technique may presumably play a part in explaining such an increase

The minor decrease in oral temperature observed in the 60–69 year group may at least in part be explained by the fact that younger subjects are more active and that an increased body temperature exists in the post ovulatory phase of the menstrual cycle in women This has been attributed to a thermogenic property of the progesterone released from the corpus luteum (Linden 1974) The decrease may also be due to sampling errors as the method of oral tempera ture recording may be unreliable in elderly people because of the difficulty of assuring adequate mouth closure (Barley & Evans 1970)

The fact that no side difference was present in the corneal temperatures of the patients with polymyalgia rheumatica indicates strongly that their ocular blood supply was intact In CRA embolism the retinal blood circulation is impaired but the considerably larger choroidal blood circulation is still intact The minor increase in corneal temperature on the affected side points to a vasodilation with increased choroidal blood flow in these patients probably initiated to compensate for the retinal hypoxia As pointed out by Mapstone (1968a) an increased blood flow in the posterior part of the eye will not increase ocular temperature as no temperature gradient exists between the vessels and their surrounding tissue in that area In the anterior part of the eye however the extravascular tissue has a lower temperature Accordingly in this part of the eye an increased blood circulation will yield an increase in temperature The minor but statistically significant increase in corneal temperature observed in these eyes indicates therefore that the increase in blood circulation which presumably exists in the posterior part of these eyes is present at least to some extent in the iris and ciliary body as well

The low corneal temperature and the marked side difference observed in active temporal arteritis may be of diagnostic importance The observed reduction in side difference during the first months of treatment until finally no side differ ence was found years after steroid treatment had started indicates that corneal temperature measurements may be of value also during the follow up of such patients The clinical distinction between CRA embolism and arteritis temporalis may sometimes be difficult However by the use of either corneal temperature measurements or dynamic tonometry (Horven 1973) or both the differentiation between these two disorders should be easy and safe

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ACUTE TRANSIENT OPHTHALMOMALACIA IN GIANT CELL ARTERITIS

Report of a Case

BY

MORTEN VERDICH and NIELS VESTI NIELSEN

Transient bilateral corneal oedema due to ocular hypotension is reported in giant cell arteritis The greatly impaired visual acuity gradually returned to normal on steroid medication A visual field defect remained in the left eye The diagnosis of giant cell arteritis was confirmed by arterial biopsy The disease involved the pericranial arteries as well as the ophthalmic artery on both sides probably also the long ciliary arteries which might explain the ophthalmomalacia Apparently only six cases are on record

Key words temporal arteritis – ophthalmomalacia – keratopathy – hypotonia – giant cell arteritis – ciliary body – ciliary blood supply

The common manifestations of temporal arteritis - which leads to severe visual impairment or blindness in one or both eyes – are ischaemic optic neuropathy or occlusion of the central retinal artery Rarely the disease causes ophthalmomalacia with transient severe visual impairment due to massive corneal oedema If the nutrition of the optic nerve and retina is unaffected vision gradually returns in step with the subsiding keratopathy

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Bettelheim (1968) presumed that the cause of the acute hypotonia was transiently reduced blood supply to the ciliary body

The object of the present communication is to describe this rare ocular symptom complex and discuss (possible) pathogenetic factors

Case report (rec No 79/75-76)

The patient was a woman aged 83 admitted because of almost total blindness The *tentative diagnosis was temporal arteritis During 1 month the patient had noticed* a painless swelling in the temporal regions Eight days before admission severe pain in the scalp and then during 1 day progressive visual impairment in both eyes without pain redness or lacrimation

In 1955 she was treated for a skin cancer on the left breast For the last 20 years she had been receiving medical treatment for an ischaemic cardiac condition but was otherwise in good health

Ocular status on admission Vision in the right eye was hand movements at 2 m in the left eye perception of light only The eyes were pale and showed normal position and motility The pupils were moderately dilated inactive to light R > L

Biomicroscopy showed severe bilateral corneal oedema The epithelium was mottled with ruptured bullae and multiple folds in Descemet s membrane There was no visible iris hyperaemia depth of anterior chamber appeared normal

Intraocular pressure measured by the method of Goldmann was 6 mmHg in the right and 5 mmHg in the left eye

Ophthalmoscopy revealed red reflexes only

General physical findings Blood pre sure 200/100 mmHg pulse rate 79/min irregular

Auscultation of heart and lungs perpetual arythmia no other abnormalities

Auscultation of the neck a brief stenotic murmur in the right major supraclavicular fossa

On the scalp two tender superficial cutaneous ulcerations were seen Nodular non tender thickening of both superficial temporal arteries no palpable pulsation in the right and only weak preauricular pul ation in the left Normal pulsation in both common carotid arteries

Laboratory findings ESR 31 mm/h Hb 9 1 mmol/l W B C 17 7 × 10^9/l serum protein 66 g/l protein electrophoresis alpha 2 20 μmol/l (normal range 5-17)

Chest radiography showed mild dilatation of the heart and pulmonary congestion

A biopsy from the right superficial temporal artery revealed giant cell arteritis

Treatment and course The patient was treated with systematic prednisone initially 60 mg daily slowly levelled off with due regard to the ESR and alpha 2 protein to a dose of 20 mg Instillation of hydrocortisone drops with terramycin and polymyxin B three times daily in both eyes

Vision remitted parallel with clearing of the corneae and increase in the tension in both eyes The cranial pain subsided simultaneously

Ocular status after 14 days Visual acuity in the right eye 6/9 in the left 6/12 with correction

Biomicroscopy showed clear corneae except for a few folds in the MD mainly in the left eye a few pigmented precipitates and a few corpuscles in the anterior aqueous The pupils reacted very slightly on maximum slit lamp illumination but contracted from 6 to 3 mm upon conjunctival application of dry pilocarpine

The ocular tension was 8/7 mmHg and 1 week later 12 mmHg in both eyes

Ophthalmodynamometry by the Bailliart method showed

Right eye 70/35 mmHg Blood pressure in right brachial artery 170/95 mmHg

Left eye 0/40 mmHg Blood pressure in left brachial artery 170/100 mmHg

Corneal sensibility (measured centrally with the Cochet & Bonnet s aesthesiometer 1°/100) was in the right eye 40 mm in the left eye 40 mm Three days later right eye 50 mm left eye 55 mm

Ophthalmoscopy of the right eye showed vascular sclerosis corresponding to the age no optic neuropathy no extravasations

The left disc was slightly pale with blurred margins and a small exudate infero temporally at the disc margin In the superior and inferior nasal quadrants the fundus was characterized by narrow retinal arterioles and ectatic venules There was a slight macular pigmentary derangement in both eyes

Goldmann perimetry of the right eye was normal (4 mm² white object) in the left eye (64 mm² white object) both upper quadrants were lost the lower temporal quadrant was restricted to 15° and the lower nasal quadrant to about 30°

Discussion

The present patient exhibited several diagnostic criteria for giant cell arteritis in particular a positive biopsy from the superficial temporal artery

Among the six cases of acute transient ophthalmomalacia in temporal arteritis on record (Haimbock (1961) one case Bettelheim (1968) two cases Goder (1968) one case Daicker & Keller (1971) one case Nagy & Juhasc (1970) one case Daicker & Keller (1971) had occasion to examine histologically one eye which was enucleated because of intractable pain They found int al in the long posterior ciliary arteries changes corresponding to giant cell arteritis with occlusion of one of these vessels It was concluded that the hypotonia was due to greatly reduced blood supply to the ciliary body followed by some formation of collaterals and perhaps increased perfusion of the anterior ciliary arteries which explained the gradual normalization of the ocular hypotonia as the ocular tension had returned to normal prior to the enucleation

Bettelheim (1968) assumed that there might be an association between the ophthalmomalacia and Horner s syndrome there being mild ptosis and enoph thalmos in both his patients However both had dilated inactive pupils due

in one to amaurosis whereas in the other patient a lesion of the ciliary ganglion was suspected

As regards the inactive dilated pupils of our patient which remained so after the corneal oedema had subsided and the visual acuity had essentially improved we assumed that this was due to an ischaemic lesion of the ciliary nerves and not to a myogenic lesion as pilocarpine could elicit contraction

The vascular supply to the anterior part of the left optic nerve was disturbed as ophthalmoscopy showed slight ischaemic papiloedema and a corresponding visual field defect

On the basis of the clinical findings it is reasonable to assume that the ocular lesion in this patient was due primarily to transiently compromised blood supply to the ciliary body caused by giant cell arteritis which had resulted in occlusion of the long posterior ciliary arteries

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STUDY OF RELATIVES OF PERSONS WITH FIBRILLOPATHIA EPITHELIOCAPSULARIS (PSEUDOEXFOLIATION OF THE LENS CAPSULE)

BY

HENRY AASVED

The frequency of fibrillopathy (pseudoexfoliation of the lens capsule) among relatives of persons with fibrillopathy was investigated Among 203 relatives over the age of 40 years belonging to 25 families fibrillopathy was found in 19 or in almost 10 % The frequency was independent of whether the proband had capsular glaucoma or normal intraocular pressure Capsular glaucoma occurred in five of the 19 relatives The results suggest that fibrillopathy may occur as a dominant hereditary trait but it may also be found sporadically

Key words fibrillopathia epitheliocapsularis – pseudoexfoliation of the lens capsule – heredity of pseudoexfoliation – capsular glaucoma

Familial occurrence of fibrillopathy (abbreviation for fibrillopathia epithelio capsularis pseudoexfoliation of the lens capsule) has been described by several authors (Vogt 1930 Gifford 1957 Amalric et al 1960 Tarkkanen 1962 Tarkkanen et al 1965 Bertelsen 1966 Sæbo 1967 Klouman 1967 Aasved 1969 Knape & Raitta 1970 and Pohjanpelta & Hurskainen 1972) In two of these re

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ports autosomal dominance was demonstrated (Tarkkanen et al 1965 Knape & Raitta 1970)

The main purpose of the present study was to investigate the frequency of fibrillopathy among relatives of persons (probands) with fibrillopathy The relation to capsular glaucoma is also discussed

Material and Methods

The persons with fibrillopathy found during an earlier mass screening in Bergen Norway (Aasved 1971a) are used a probands in the present study Thus the probands did not themselves apply to a physician for trouble with their eyes In the present study as many as possible of the probands siblings children cousins nieces and nephews above the age of 30 were examined None of the probands relatives in earlier generations were alive but information on any known cases of glaucoma in the family was obtained

In the case of three of the relatives in this material data have been obtained from other ophthalmologists all the others have been examined by the author A Haag Streit 900 slit lamp was employed and the pupils were dilated with either metaoxedrin 10 % or cyclopentolate chloride (Cyclogyl®) The intraocular pressure was measured with a Schiotz weight tonometer If the intraocular pressure was found to be higher than 4 5/5 5 supplementary examinations were made consisting of tonography and water drinking tests The following criteria for glaucoma were employed Increased intraocular pressure combined with excavation of the optic discs to the edge and/or visual field defects characteristic of glaucoma or intraocular pressure above 25 mmHg combined with outflow facility less than 0 12 mm³/min/mmHg and/or increase of i o p in water drinking test of more than 10 mmHg

A total of 225 persons belonging to 25 families were examined In the further analysis all the 22 persons between the ages of 30 and 39 years were eliminated None of these had fibrillopathy or glaucoma The material used in further analysis thus consisted of 203 persons over the age of 40 years not including the probands A number of relatives were not called in for examination as they lived at considerable distances from the place of examination Of those called in 11 did not attend examination

The size of the families varied considerably In 15 families four or more relatives were examined the highest number being 37 whereas in 10 families there were less than four relatives

In some of the analyses the material was divided into two groups one in which

Table I
Age and sex distribution of the relatives examined

	Years					Total	Mean age
	40–49	50–59	60–69	70–79	80–89		
	No exam	No exam	No exam	No exam	No exam	No exam	Years
	Proband with capsular glaucoma						
Women	5	10	14	10	2	41	63 [illegible]
Men	9	11	9	8	1	38	59 [illegible]
Total	14	21	23	18	3	79	61 5
	Proband with fibrillopathy and normal i o p						
Women	15	25	15	13	3	71	59 7
Men	15	10	13	10	5	53	60 6
Total	30	35	28	23	8	124	59 8
	Total material						
Women	20	35	29	23	5	112	60 7
Men	24	21	22	18	6	91	60 2
Total	44	56	51	41	11	203	60 5

the proband had capsular glaucoma and the other in which the proband had fibrillopathy accompanied by normal intraocular pressure

The age distribution appears from Table I The average age in the total material was 60 5 years and was approximately the same for men and women

Results

The occurrence of fibrillopathy among the relatives examined appears from Table II The bottom part of the Table shows the number of relatives suffering from glaucoma – in all cases capsular glaucoma

All in all fibrillopathy was found in 19 of 203 relatives or in almost 10 % The difference between women and men is not statistically significant ($P > 0\ 30$)

Table V
Fibrillopathy with or without capsular glaucoma demonstrated in direct descendants of patients with glaucoma

Family no	No children examined	No with fibrillopathy and normal i o p	No with capsular glaucoma
I	3	0	1
II	2	1	0
III	4	0	1
IV	1	0	0
V	5	1	2
VI	1	0	1
VII	4	2	0
VII	2	0	0
VII	3	0	0
VII	2	1	0
VIII	2	0	0
VIII	2	0	0
VIII	4	0	0
8 families	35	5	5

which no fibrillopathy was found among the relatives glaucoma is known to have occurred in the preceding generation in all three cases in the father of the proband

The frequency of fibrillopathy and capsular glaucoma among the direct descendants of patients with glaucoma appears from Table V A total of 35 descendants of 13 patients with glaucoma have been examined these 35 being distributed over eight families All in all fibrillopathy without glaucoma was found in five descendants and capsular glaucoma in five others These 10 were among 21 direct descendants of seven patients with glaucoma no such findings being made among the 14 descendants of the other six patients with glaucoma

Comments

In this study fibrillopathy was demonstrated among almost 10 % of the examined relatives of persons with a previous history of fibrillopathy This accords

Table I
Age and sex distribution of the relatives examined

	Years					Total	Mean age
	40–49	50–59	60–69	70–79	[illegible]		
	No exam.	No exam.	No exam.	No exam.	[illegible]	[illegible]	[illegible]
Proband with capsular glaucoma							
Women	5	10	14	1	–		
Men	9	11	9	[illegible]		[illegible]	[illegible]
Total	14	21	2[illegible]	1	[illegible]	–	
Proband with fib[illegible]							
Women	15	[illegible]	[illegible]		[illegible]	–	
Men	15	19		1	[illegible]		–
Total	30	[illegible]	[illegible]	[illegible]		[illegible]	
Total material							
Women	[illegible]	[illegible]	[illegible]	[illegible]			
Men	24	[illegible]	[illegible]				
Total	44	[illegible]	[illegible]			[illegible]	–

The frequency also appears to be statistically independent of whether the proband had capsular glaucoma or normal intraocular pressure ($P > 0.30$)

Glaucoma occurred in five of the 19 relatives with fibrillopathy (26 %) and the frequency does not appear to depend on whether the proband had capsular glaucoma or normal intraocular pressure

The frequency of fibrillopathy among relatives in the different age groups appears from Table III As the findings appeared to be independent of sex and of whether the proband had capsular glaucoma or normal intraocular pressure these groups were combined As the number of relatives examined in each age group was rather small the frequency varies somewhat but shows as might be expected an increasing tendency with increasing age

Fibrillopathy among relatives was found in nine families of the total of 25 This may have some connection with the number of relatives examined in the two groups

The average number of relatives per family was 10 7 but the number was far higher in the nine families in which fibrillopathy was found among relatives (15 3) than in the other 16 families (4 1)

The occurrence of fibrillopathy or of capsular glaucoma in the different categories of relatives appears from Table IV The frequency among nieces/

Table II

Occurrence of fibrillopathy among relatives

	Proband with capsular glaucoma			Proband with normal i o p			Total		
	No exam	No with fib	Per cent	No exam	No with fib	Per cent	No exam	No with fib	Per cent
Women	41	4	9 8	71	7	9 9	112	11	9 8
Men	38	2	5 3	53	6	11 3	91	8	8 8
Total	79	6	7 6	124	13	10 5	203	19	9 4
No of relatives with capsular glaucoma		1			4				

Table III
Frequency of relatives with fibrillopathy in different age groups

Age groups years	No relatives examined	No relatives with fibrillopathy	Per cent (rounded off)
40–49	44	0	–
50–59	56	3	5
60–69	51	8	16
70–79	41	3	7
80–89	11	5	45
Total	203	19	9.4

nephews is considerably lower (5 %) than in the other groups In these other groups the differences may have occurred by chance ($P > 0.30$)

Fibrillopathy was found in the same generation as the proband (siblings cousins) in five families In four of these glaucoma is known to have occurred in the preceding generation

In four families fibrillopathy was demonstrated in the generation following that of the proband (child niece nephew) In one of these families glaucoma is known to have occurred in the preceding generation In three of the families in

Table IV
Occurrence of fibrillopathy and capsular glaucoma in the various categories of relatives

	No persons examined	Total no with fibrillopathy	No with capsular glaucoma
Children	12	1	0
Siblings	36	4	2
Niece/nephew	119	6	0
Paternal cousins	12	4	1
Maternal cousins	24	4	2
Total	203	19	5

Table V
Fibrillopathy with or without capsular glaucoma demonstrated in direct descendants of patients with glaucoma

Family no	No children examined	No with fibrillopathy and normal i o p	No with capsular glaucoma
I	3	0	1
II	2	1	0
III	4	0	1
IV	1	0	0
V	5	1	2
VI	1	0	1
VII	4	2	0
VII	2	0	0
VII	3	0	0
VII	2	1	0
VIII	2	0	0
VIII	2	0	0
VIII	4	0	0
8 families	35	5	5

which no fibrillopathy was found among the relatives glaucoma is known to have occurred in the preceding generation in all three cases in the father of the proband

The frequency of fibrillopathy and capsular glaucoma among the direct descendants of patients with glaucoma appears from Table V A total of 35 descendants of 13 patients with glaucoma have been examined these 35 being distributed over eight families All in all fibrillopathy without glaucoma was found in five descendants and capsular glaucoma in five others These 10 were among 21 direct descendants of seven patients with glaucoma no such findings being made among the 14 descendants of the other six patients with glaucoma

Comments

In this study fibrillopathy was demonstrated among almost 10 % of the examined relatives of persons with a previous history of fibrillopathy This accords

well with the results of comparable earlier studies (Pohjanpelta & Hurskainen 1972) The frequency is considerably higher than that found on mass screening of persons above the age of 40 carried out in the same region by the author (approx 1 % Aasved 1971a) This difference in frequency must be assumed to be due to an hereditary factor

In this study no fibrillopathy was found in four of 11 families in which seven or more of the relatives of the proband were examined Thus fibrillopathy also may occur sporadically with no evidence of a hereditary factor

The study revealed fibrillopathy in two generations in four families In addition glaucoma is known to have occurred in a preceding generation in four of the five families in which fibrillopathy was demonstrated in the same generation as the proband If this earlier glaucoma is taken to be capsular glaucoma this means that there is information and/or findings in two successive generations in eight of the nine families in which fibrillopathy was found among the relatives examined It can be noted that glaucoma was known to have occurred in the preceding generation in only three of the 16 families in which fibrillopathy was found only in the proband

As in an earlier study glaucoma was found in approximately 1/4 of the persons with fibrillopathy (Aasved 1971b) Within the same family however one may find a mixture of persons with capsular glaucoma and persons with fibrillopathy associated with normal intraocular pressure In this study this was the case in six of the nine families in which fibrillopathy was found among the relatives

The results suggest that fibrillopathy may occur as a dominant hereditary trait but that the penetration appears to vary somewhat

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ORBITAL TUMOURS IN INFANCY

An analysis of Danish cases from 1943–1962

BY

P ELDRUP-JORGENSEN and HANS FLEDELIUS

A Danish nation wide 20 year series of orbital tumours in infancy is reported making up a total of 80 histopathologically verified cases Secondary tumours were not included except for a few cases where the initial manifestation of the disease was an orbital mass suggesting a primary tumour of the orbit

A scant half of the series were benign choristomas Otherwise there was a considerable share of more serious diseases often threatening vision and/or life thus ten embryonal sarcomas and nine optic nerve gliomas were found The malignancies made up a total of 17 cases

Because of the many medical specialities necessary for prompt diagnosis and efficient therapy a centralization of the more serious orbital cases is felt mandatory Considering the rather low overall frequency of orbital tumours a centralization will not constitute a load numerically an estimate for Denmark (population 5 million) is about five infantile cases a year

Key words orbital tumours in Denmark – infancy – primary and secondary tumours – malignancy – embryonal sarcoma – optic nerve glioma – choristoma – centralization

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In 1974 four Danish infants were treated – without success – for embryonal sarcoma of the orbit Our review of the histories and the treatment given has left us with the impression that such – rare – cases should be centralized Only thus will it be possible to co ordinate the various medical specialities that are necessary for prompt diagnosis and optimum treatment

To get an idea of the implications of centralization two studies were under taken on a national scale 1) A review of the whole spectrum of orbital tumours of childhood and 2) a review solely comprising the orbital rhabdomyosarcomas and embryonal sarcomas This report deals with the first of the two studies The results obtained are considered to be of wider interest because of the great difficulty in establishing the true frequencies of the various orbital tumours in infancy

Material and Methods

The material has been collected by one of the authors (P E J) as part of a major investigation into primary orbital (i e located behind the orbital septum) tumours in Denmark 1943–1967 with a population then of 4 3 million To make the survey nation wide the files of the larger Danish eye departments the radium centres and the neurosurgical departments were closely examined Orbital cases with tentative diagnoses were eliminated only those patients with surgical and histopathological confirmation were included In a few cases the tumours later proved to be metastases (neuroblastoma two cases) or manifestations of generalized disease (leukemia lym phosarcomatosis four cases) they were however retained within the survey because the presenting sign was that of an orbital tumour Cases representing local extensions from tumours in adjacent regions e g from eye (retinoblastomas) and eyelids were omitted from the series Part of the material covering all age groups has already been published (Eldrup Jorgensen 1970) as a preliminary report

The present review deals with 80 paediatric cases belonging to the 0–15 year age group We have reviewed and classified in the Eye Pathology Institute the histopathological sections from almost all lesions included (75/80) Paraffin sections were stained routinely with haematoxylin eosin supplemented however with special staining methods where relevant In five (undoubtedly benign) cases it was necessary to use the original pathology descriptions because material or sections were no longer available

Results

Table I shows the classification of the 80 microscopically verified orbital tumours 17 of which were considered malignant (shown in brackets) One case of optic nerve glioma which caused the death of the patient was included among the malignancies – although with reservation.

Table I
Relative frequencies of orbital tumours in infancy

Tumours with origin in	The present Danish series n = 80	Porterfield (1962 modified) n = 214	Crawford (1967 modified) n = 53
Lacrimal gland or other epithelial tissue	2	6 (3)	
Fibrous tissue/xanthomatous and histiocytic tissue		2	
Adipose tissue	3	3	
Embryonal mesenchymal and muscle tissue	10 (10)	66 (65)	5 (5)
Blood and lymph vessels	6	28	11
Bone and cartilage	1		
Peripheral and autonomic nerves	3	7	1
Optic nerve	9 (1)	39 (*3)	4
Choristomas (dermoid cyst teratoma)	39	13	4
Melanogenic system			
Haematopoietic or lymphoid tissue	4 (4)	12 (12)	12 (12)
Inflammatory tumour like lesions (pseudotumour)	1	16	3
Secondary tumours	2 (2)	3 (3)	13 (10)
Others		14 (11)	

Histopathological classification of Danish primary orbital tumours in infancy 1943–1962 (left column) The number of malignancies is shown in brackets The asterisk beside optic nerve glioma designates known fatal cases (cf Discussion) For comparison, Porterfield's histopathological series (1962) and Crawford's clinical series (1967) modified by the present authors to fit into the classification of the Danish series are given

By far the largest single group in Table I consisted of the benign *choristomas* comprising 38 dermoid cysts and one teratoma According to the descriptions given by the ophthalmic surgeons half the choristomas were located in the orbit proper the other half being described as orbito palpebral

Next in frequency were two clinically far more serious groups *Embryonal sarcoma + rhabdomyosarcoma* (10 cases) and *optic nerve glioma* (9 cases) The orbital *sarcomas* will be the subject of a later paper we restrict ourselves here by mentioning that only two infants of the group have survived

The *optic nerve gliomas* (primary orbital) have all been evaluated as benign according to histological features Their tendency to invasive growth after incomplete removal proved fatal however for one infant in the group Another patient died of a phaeochromocytoma 23 years after radical orbital surgery (Fledelius 1975) The remaining seven are still living Von Recklinghausen s disease was diagnosed in three of the nine cases with optic nerve glioma

Orbitally located *neurofibromata* as part of von Recklinghausen s disease occurred in three other infants of the series quoted in the *peripheral and autonomic* nerve group of Table I Exenteration of the orbit, and excision of periorbital tumour tissue were necessary in two of these cases due to the advanced stage prior to surgery

There were six benign *vascular* tumours (haemangioma haemangio endothelioma) Three orbital masses were the initial solitary manifestations (so called chloromas) of stem cell *leukaemias* fatal after a few months One of these had constant high eosinophilic counts in the blood and eosinophilic leukaemia was suggested One infant died of a generalized *lymphosarcomatosis* which started in the orbit clinically The two secondary tumours of Table I were orbital metastases from neuroblastomas both infants died in spite of extensive radiotherapy

The remaining cases – all benign – are listed in the following order (cf Table I) One pleomorphic adenoma of the lacrimal gland one benign adenomatous epithelial proliferation (from sweat gland or lacrimal gland) three (fibro) lipomas one osteoma or osteofibroma one inflammatory sclerosing pseudo tumor at the site of the lacrimal gland

As already stated the number of *malignant* orbital tumours in this 20 year series was 17 This means about one case a year in Denmark (population 4 9 million in the sampling period) see also Table II The later clinical history (in terms of survival for more than 15 years after treatment) has been mentioned above for each of the diagnostic groups included

Table II
Incidence of orbital tumours in infancy

	Population of drainage area (all ages)	Period	Number in paediatric age group	
			Orbital tumours per year	Orbital malignancies per year
Denmark (on a national scale)	4.3 mill	1943–1962	4	1
Canada (Toronto area)	about 3 mill	1942–1965	3	1
Parts of U S + foreign cases (Armed Forces Institute of Pathology)	?	–1961	estimate 12–18	estimate 5–10

Annual number of orbital tumours in the paediatric age group (0–15 years) in Denmark and Canada based on the present series and the data published by Crawford (1967 cf Table I) For the third material included (Porterfield 1962) only estimates can be given

Discussion

The orbit is among the six most common sites of malignant disease in child hood (Dargeon 1960) a fact which is reflected in the relative predominance of the paediatric age classes in most published orbital tumour series (e g Porterfield 1962 Eldrup Jørgensen 1970)

The overall incidence of orbital tumours is however low It is therefore difficult to obtain representative data and to establish the true frequencies of the various tumours

Most of this information is compiled from smaller series or deduced from larger series often with no clear division according to age groups Further more the results obtained largely depend upon which medical speciality is in volved This has been convincingly demonstrated by Reese (1963) when com paring three orbital series ophthalmological histopathological and radiological

The Danish medical system – and the small size of our country – has en abled us to carry out a representative 20 year nation wide review of orbital

tumours in infancy (Table I) Since a large proportion of suspected orbital lesions can be clarified only microscopically we have based the present series solely on histopathological criteria The same line was followed by Porterfield (1962) whose data from 214 (selected) paediatric orbital cases has been included in Table I Porterfield s classification differs a little from the more topical classification chosen by us and we are responsible for the (slight) rearrangement of Porterfield s findings

It was more difficult to fit the *clinical* series of Crawford (1967 Canada) into the scheme of Table I The primary aim of Crawford s report comprising 257 infants with ocular protrusion was to show the whole spectrum of underlying disease met with in a large paediatric hospital and no scope was left for the discussion of histopathological features Although only one fifth (54/257) of the Canadian sample could be considered as true tumours we included this fraction in Table I because the series (in contrast to Porterfield s) has been regarded as representative of a well defined geographical area (Crawford 1975) On the other hand the general paediatric aspect gave rise to a comparatively large proportion of *secondary* tumours (14/54) most of which would have been omitted according to the criteria of the other two series in the Table We excluded only one of Crawford s secondary cases an orbital extension of a retinoblastoma thereby reducing his sample to 53

The differences between the three series in Table I are due to the somewhat different delimitations already touched upon Of the two histopathologically based series the larger (Porterfield s) cannot be regarded as representative as the author himself points out there is a skewness towards more severe (malignant) and/or problem cases This probably explains why the number of dermoid cysts does not reach the level of our Danish series Dermoid cysts present no problems to local pathologists and are not referred to an ophthalmic pathology centre (here A F I P) The shortcoming of the Danish – truly representative – 20 year series is its small size For this reason we added the two other materials in Table I in our opinion a reasonable overall impression of orbital tumours in infancy is thereby obtained

Haemangiomas are usually considered the most frequent of all orbital tumours (Reese 1963) but in Table I (with paediatric cases) they are outnumbered by the various malignancies Concerning the two histopathological series this is easily explained by the fact that surgical treatment (and microscopical examination) of the admittedly congenital haemangiomas is usually postponed until adult age because in infancy the ocular protrusion is often only slight or absent (Iliff & Ossofsky 1962)

The striking feature of Table I is the high proportion of malignant disease of which embryonal (rhabdomyo) sarcomas are the most frequent they run

a stormy course with an extremely high mortality and demand immediate treatment whenever suspected The optic nerve gliomas – although not truly malignant histologically – threaten vision and life if not excised early and completely The leukaemic disorders call for radio and chemotherapy the evaluation of paediatricians is essential also for many other orbital cases in infancy

It is not our intention to discuss the therapeutic aspects in further detail here We merely wish to emphasize the need to centralize infantile orbital cases suspected of malignancy – to avoid the otherwise inevitable sometimes fatal doctor s delay due to the many branches of medicine involved in diagnosis and treatment (neurosurgery ophthalmology otorhino laryngology paediatrics pathology plastic surgery radiotherapy) In the case of Denmark such views were prevalent when motivating the establishment of a Danish ophthalmic tumour centre with consultative and coordinating functions (since 1964 cf Gregersen 1975)

Finally a short comment on Table II the rare occurrence of malignant orbital tumours in childhood is an additional reason for centralization It will otherwise not be possible to collect experience and improve results

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SCLERAL PREPLAQUES AND PLAQUES IN ESKIMOS

The incidence in West Greenland Eskimos compared with that in Copenhagen Caucasians

BY

M. S. NORN

Examination of the eyes of 519 Eskimos in West Greenland revealed preplaques (grey, ill defined translucent bands on the sclera anterior to the insertion site of the horizontal recti) in 4 % and scleral plaques (well defined greyish translucency of the sclera) in 2 %. The incidence was found to rise with increasing age.
Preplaques are more rarely present in Eskimos than in Copenhagen Caucasians, where the incidence of plaques is the same. The cause of this ethnic difference is discussed.

Key words: sclera – scleral plaques – preplaques – Eskimos – Greenland – racial difference.

In some elderly individuals a vertical greyish streak may be seen on the sclera in front of one or more of the horizontal muscles. This phenomenon was first described by the author in 1973. I evidenced that the phenomenon might be due to increased transparency of the sclera and that it is a precursor of the well known hyaline scleral senile plaques.

My examinations concerning scleral preplaques and plaques were carried

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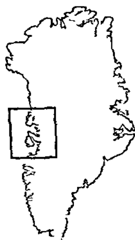


Fig 1
West Greenland from Upernavik to Christianshåb

out on Danish Caucasian patients in an Ophthalmic out patient department and an ophthalmic practice in Copenhagen within the period from October 1971 to October 1973

In July 1975 as ophthalmologic adviser I had occasion to subject Danish Eskimos in West Greenland to similar examinations

Material

The investigation comprised all the Eskimos referred in 1975 to the West Greenland medical districts (Fig 1) for eye examination from Upernavik in the north (72 4 N lat) to Christianshab and Egedesminde in the south (68 4 N lat) This Eskimo material has been compared with the results of my previous studies on Copenhagen Caucasians (Norn 1973 1974a b)

Definition

By scleral *preplaque* we understand an ill defined grossly visible translucent scleral area It is about 1 mm broad runs a vertical course in front of the site of insertion of a horizontal eye muscle in its entire breadth



Fig 2
Scleral preplaque and plaque examined in the slit lamp with diffuse light

A scleral *plaque* is a well defined greyish translucent scleral area most easily recognizable in the slit lamp The plaque is round oval or irregular situated in front of the insertion of an eye muscle (Figs 2 and 3) The trans parency of the superficial part of the sclera allows the uvea to shine through

Method

Preplaques are detectable by gross examination with an ordinary pencil lamp A scleral translucency is characterized as a preplaque if a distinct greyish



Fig 3
Scleral plaque examined in the slit lamp with oblique slit

band is seen Irregular translucent areas following scleritis or diathermy (operation for retinal detachment) are not included

A scleral plaque is visible in the slit lamp preferably with indirect lighting and is seen as a clear cut border opposite to the slit (Norn 1974c)

The examinations in Greenland were performed with a hand held Kowa *slit lamp while a Haag Streit slit lamp 900 was used for the examinations* in Copenhagen In both cases direct and indirect lighting were used with ten times magnification The two slit lamps are equal in illumination intensity and slit and are therefore equally suitable for the detection of plaques

Results

Preplaques occur in Eskimo eyes though more rarely than in Caucasian eyes The incidence rises with increasing age (Table I)

The difference is significant within the age groups below 60 60–69 and 70–79 ($P < 0\ 05$ $< 0\ 01$ and $< 0\ 001$ respectively)

Scleral plaques also occur in Eskimos The incidence is the same as among Caucasians (Table II) and rises with increasing age The plaque morphology is the same as in Caucasians vertically oval and circular plaques being the most frequent (14 vertically oval and 13 circular in altogether 10 subjects)

Table I

Incidence of preplaques in Eskimos compared with that in Caucasians in different age groups

Age	Incidence in %		Number of subjects examined	
	Eskimos	Caucasians	Eskimos	Caucasians
< 60	0 3	4	369	53
60–69	8	30	72	66
70–79	17	43	65	136
80–89	25	54	12	61
≧ 90	100	100	1	3
Total	(4 9)	(37)	519	319

Table II
Incidence of scleral plaques in Eskimos compared with that in Caucasians in different age groups

Age	Incidence in %		Number of subjects examined	
	Eskimos	Caucasians	Eskimos	Caucasians
< 60	0	1	369	181
60–69	3	3	72	247
70–79	8	10	65	405
≥ 80	23	25	13	253
Total	(1 9)	(10)	519	1086

In Caucasians scleral plaques and preplaques are most often located medially (68 and 63 % respectively) more rarely laterally or equally distributed The medial location seems to be less predominant in Eskimos (40 and 41 % respectively) However difference is not significant

The sex incidence is the same in the two races

Discussion

The two materials were examined by the same investigator with the same technique concerning preplaques (ordinary pencil lamp) and comparable techniques concerning scleral plaques (Kowa and Haag Streit slit lamp)

The investigation showed preplaques to be rarer among West Greenland Eskimos than among Copenhagen Caucasians

The West Greenland Eskimos are often of mixed descent (Skeller 1954) A comparison between pure Eskimos and pure Caucasians would probably disclose greater differences

The incidence of scleral plaques is the same in the two ethnic groups

Preplaques are considered to be precursors of plaques

The preliminary stage of proper scleral plaques may be shorter in Eskimos while scleral plaques develop at the same point of time in the two races

Why are preplaques more rarely found in Es imos? Different possibilities may be suggested

Arteriosclerosis is rare among Fskimos This is due to the fatty content of the diet (long chained poly unsaturated fatty acids? Bang & Dyerberg 1975) Arteriosclerosis of the anterior ciliary arteries will in particular act on the zone in front of the insertion site of the horizontal muscles and thus perhaps provoke preplaques

Pterygium and pinguecula (Skeller 1949 Clemmesen 1973) occur more frequently in Fskimos In the fairly pronounced cases these phenomena may possibly conceal preplaques However no correlation was noticed in the Cau casian series (Norn 1973) between preplaques and such formations and in the Eskimo series under review only a few cases of thick possibly lobulated growths were seen

Pigmentation is more pronounced in Eskimos Irregularly located conjunc tival pigmentations were often found but hardly situated so as to conceal preplaques On the contrary the increased uveal pigmentation rather tends towards easier recognition of preplaques

The arctic climate predisposes to desiccation. According to one of the theo ries regarding the development of plaques however desiccation is liable to promote their formation

Elderly Eskimos probably read less than elderly Caucasians The conver gence movement exposes the sclera to wear particularly so off the medial recti where the majority of preplaques are situated

There may be other factors involved (thickness of sclera or conjunctiva?)

The cause of the rare occurrence of scleral preplaques in Eskimos is as yet obscure

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PARALIMBAL SCLEROMALACIA

So called spontaneous scleral intercalary perforation

BY

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A case of unilateral paralimbal scleromalacia (so called spontaneous scleral intercalary perforation) is presented The otherwise healthy patient was followed over a period of more than 10 years Two years before the condition was diagnosed the patient had an attack of scleritis in the same eye The disease was complicated by keratitis and resulted in an eye with light perception only Scleral tissue from the defect was examined histologically

Key words paralimbal scleromalacia – spontaneous scleral intercalary perforation – histological examination – sclera – keratitis

Paralimbal scleromalacia is a rare ocular disorder According to Cappin & Allen (1973) 16 cases have been reported in the world literature The first cases were orginally described by van der Hoeve (1934) as scleromalacia perforans Later the disease was distinguished from this condition and from necrotizing nodular scleritis by Franceschetti & Bischler (1950) François (1951) suggested the name spontaneous scleral intercalary perforation indicating that there is no proof that the disease is a necrotic affection as is the case with

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scleromalacia perforans Only a few histological studies have yet been de scribed

This is a report of a patient with spontaneous scleral intercalary perforation without any systemic associations

Case report

The patient is a 31 year old woman previously in good health without any history of joint affections The patient had a goitre for several years but showed euthyroid

At the age of 18 years (1961) she had an attack of redness in the lower half of the right bulbus diagnosed as scleritis and treated with X rays and systemic corti costeroids

Two years later (1963) a white partly pellucid cystoid tumour was observed in the right eye at the 4 to 7 o clock position at the limbus No normal scleral tissue was seen between the lesion and the cornea The absence of vascularity was striking (Fig 1) No irisprolapse was seen the pupil was not ectopic Gonioscopy revealed a normal open angle with some narrowing under the cyst The cornea lens and fundus were normal There was no intraocular inflammation Tension was 8 mmHg on applanation Vision was 0 67 with -3 00 cyl 100° There was no tenderness of the eye The left eye was normal Two months later the cyst had increased in size and gonioscopy revealed a fine grey line situated on Schwalbe s line Subconjunctival fluorescein injection showed no passage into the cyst and injection into the cyst itself showed no passage to the anterior chamber The cyst was removed The under

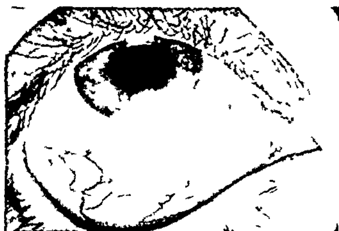


Fig 1
Paralimbal scleromalacia in the right eye when the patient was 20 years old A white cystoid tumour without injection is situated in the lower half of the bulbus



Fig 2

Paralimbal scleromalacia when the patient was 31 years old White cysts are still present at the 5 and 7 o clock positions The cornea is oedematous with white opacities

lying sclera was extremely thin but there was no perforation Microscopy (Eye Path Inst No 481/63) of a small piece of the sclera from the bottom of the cyst showed cicatricial tissue with derangement of collagen fibres and few cell nuclei Those present were degenerated or necrotic There was no active scleral inflammation The arterioles seen were normal The conjunctiva and the episclera showed unspecific inflammation. There were no fungi found by PAS staining

One year later (1964) small crystalline deposits were observed in the corneal stroma at the base of the cyst which had reformed Gonioscopy showed a furrow at the 4 to 7 o clock position The patient underwent two conjunctival operations owing to retraction of the conjunctiva

Five years later (1970) the patient noticed redness and decreasing vision of the right eye Visual acuity was found to be counting fingers at 2 meters Biomicroscopy showed corneal oedema but no intraocular inflammation

At the age of 30 years – 10 years after the first admission – the patient was successfully treated elsewhere for a keratitis of the right eye

At the last admission when the patient was 31 years old (1974) the visual acuity was reduced to light perception with projection Avascular blue grey cysts were still present at the 4 and 7 o clock positions but the uveal tissue did not protude into the blebs The entire cornea was thick and oedematous with two white opacities in the lower half (Fig 2) The iris and lens were almost invisible The tension was normal on palpation Intraocular inflammation was not present

General examination There was no swelling of the joints The patient had a moderate diffuse enlargement of the thyroid gland but was found euthyroid

Table 1

	Arkle & Ingram	Bonamour & Bonnet	Cappin & Allen	Eber	Franceschetti & Bischler	van der Hoeve	Mitter	Nirankari et al	Sivasubramaniam & Mutucumarana	Sorensen	Turtz	Zygulska Machowa & Osterczy Sliwinska
Age	34	21	75	74	23	69	24	20	unknown (adult)	20	38	51
Sex	female	male	male	male	female	male	male	male	male	female	female	male
Joint affection	–	–	–	+	?	–	+	–	–	–	–	–
Binocularity	unilateral	bilateral	unilateral	unilateral	unilateral	bilateral	unilateral	unilateral	bilateral	unilateral	bilateral	unilateral
History of pain and redness	–	–	–	+ (trichiasis)	+	++	+	+	++	+	++	–
Evolution	slow (years)	slow (years)	slow (years)	slow (years)	rapid (days)	slow (years)	slow (months)	slow (years)	slow (years)	slow (years)	slow (months)	?

Position	*temporal* limbus	upper limbus	upper limbus	upper limbus	?	upper and nasal limbus	lower limbus	upper limbus	upper *limbus*	lower *limbus*	upper *limbus*	?
Irisprolapse	–	–	+	–	+	in one eye	–	–	++	–	–	?
Keratitis	–	–	–	–	–	–	–	+	one cornea white	+	in one eye	?
Lues	?	?	WR pos TPI neg	?	?	?	WR neg	?	Kahn *test* neg	?	WR *neg*	cerebro spinal lues
Follow up	3 years	12 years	–	3 years	–	2 months	3 years	10 months	–	11 years	2 years	1 year
Prognosis	good	poor	poor	good	good	good	poor	poor	poor	poor	good	good

Laboratory investigations No sign of hyperthyroidism Blood cholesterol was normal Erythrocyte sedimentation rate was 5 mm/h LE factor and Rose Waaler's test were negative

Discussion

Paralimbal scleromalacia was described as an entity by Franceschetti & Bischler (1950) The clinical features are outlined in the Table which summarizes the data for 11 cases collected from the literature The disorder occurs in otherwise healthy adults of either sex and sometimes bilaterally

It is characterized by the development of a paralimbal cystic degeneration which may lead to spontaneous scleral perforation The appearance may be that of a filtering bleb with or without uveal prolapse There may be no signs of ocular inflammation but episodes of inflammation occurred in four of the seven unequivocal cases mentioned by Duke Elder & Leigh (1965) In our case as well as in the one reported by Nirankari Parkash & Singh (1962) an acute scleritis was described prior to the development of the cyst The cornea is often clear although peripheral opacities or deposits may occur corresponding to the cyst The prognosis is usually said to be good However in five of the cases in the Table the prognosis was poor owing to keratitis corneal opacities or folds in Descemet's membrane Anterior uveitis has only been reported in one case (Nirankari et al 1962)

The final outcome in the present case was bad due to central corneal opacity which has been reported in only one other case (Sivasubramaniam & Mutucumarana 1960) The localization of the lesion has most often been along the upper part of the limbus but in our case as well as in that of Mitter (1948) the lesion was sited inferiorly

Histological studies of paralimbal scleromalacia are few and only briefly described (Eber 1934 van der Hoeve 1934)

The biopsy material from the present case revealed no necrotic or inflammatory lesions as found e g in scleromalacia perforans

One must agree with Franceschetti & Bischler (1950) that these patients show a characteristic and peculiar ocular disorder which make it reasonable to group them as a clinical entity But it is still doubtful whether these relatively few patients have more than the clinical aspect in common The pathogenesis is obscure At first a cyst develops which from the injection experiments with fluorescein performed on our patient shows no communication to the anterior chamber nor to the subconjunctival space Later the "scleromalacia" may lead to perforation

Cystic changes are described as occuring in marginal degenerations with thinning of the normal limbal structures and in extensive degenerations (lipid or hyalin) These cysts are quite superficial in location and are often covered by a thin conjunctiva Limbus girdle or arcus senilis are frequently present indicating other degenerative processes It may be considered an open question whether these cysts in degenerative tissue have anything in common with paralimbal scleromalacia

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EYE INJURIES CAUSED BY TEAR GAS HAND WEAPONS

BY

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Six patients hospitalized by eye injuries caused by tear gas hand weapons are presented Five of the lesions resulted from a short distance shot of aerosol irritant projectors Severe swelling and bloodshot of the lids and conjunctiva occurred in all cases Epithelial defects swelling and blurring of the parenchym were observed in the cornea The treatment of each case took weeks even months Such an injury can because of scars in the cornea permanently lower the visual acuity

Key words tear gas – tear gas weapons – chloroacetophenone – eye injuries

Chloroacetophenone (CN) is the lacrimatory agent currently used in almost all tear gas weapons CN causes two different kinds of reactions at low concentrations a lacrimogenic reversible effect on the corneal nerve endings and at high concentrations an injurious denaturing difficulty reversible effect on eye tissues (Grant 1974)

Until the introduction of the nonexplosive weapon type in the late 1960s most of the weapons employed an explosive charge to propel and disperse the tear gas material The typical small hand weapon fires a cartridges containing solid CN and the explosive charge These materials are sealed into the cartridge with a wadding composed of cork wax rubber cardboard or

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synthetic material Upon firing a suspension of fine particles results similar to an aerosol (Hoffmann 1965 Levine & Stahl 1968)

Since the introduction of the explosive tear gas weapons numerous incidents involving serious damage to the eye as a result of the discharge from tear gas weapons have occurred (Midtbo 1964 Hoffmann 1965 1967 Levine & Stahl 1968 Laibson & Oconor 1970) These are particularly clearly reviewed and tabulated by MacLeod (1969)

In most cases the eye injury seem to have been associated with exposure to an explosive type weapon close to the face Under these circumstances ocular damage was caused by the lacrimator itself associated with trauma from explosive materials wadding undispersed solids and thermal insult (MacLeod 1969)

The nonexplosive weapon type on the contrary does not utilize explosives solid materials or heat The typical solvent spray weapon type consists of a pressurized aerosol canister containing about 1 % CN an organic solvent system and propellant gas The mixture is released as a spray of small liquid droplets directed in a stream that may travel up to 6 m Most of the solvents evaporate from the droplets leaving the less votile residue where CN is considerably more concentrated than the 1 % in the original mixture (Grant 1974)

Clinical observations on the ocular effects of the solvent spray type of tear gas weapon are remarkably rare in scientific literature Rose (1969) described 12 patients 3 of whom had a longlasting epithelial injury in cornea and conjunctiva after a close range shot by aerosol irritant projectors The other 9 of the epithelial injuries in cornea and conjunctiva healed in 72 h

This report will discuss 6 moderate injuries to the eye by tear gas hand weapons – 5 by aerosol irritant projectors and 1 by a tear gas pistol The patients were all hospitalized in Turku University Central Hospital Department of Ophthalmology during the years 1972–1974

Case 1

A 26 year old drunken metal worker who was arrested by the police Because of violence he was incapacitated with a tear gas spray held at a distance of less than one meter The patient sought ophthalmological help 12 hours after the attack because of sharp pain and photophopia in his left eye

Examination of the left eye revealed moderate edema and erythema of the left upper and lower lids Purulent exudate from which Staphylococcus aureus was isolated was abundant on the erythematous and swollen conjunctiva Visual acuity in the eye involved was 0.5

Slit lamp examination of the left eye showed a large epithelial abrasion in the lower half of the cornea and conjunctiva marked stromal edema and many folds in the Descemet s membrane There was some cells and flare in the anterior chamber Iris vessels were dilatated and there was an irritative miosis

Atropin and chloramphenicol were applied topically On the second day the patient moved to an other hospital where atropin and chloramphenicol were continued He was discharged two weeks after the injury

Punctate epithelial defects in the cornea folds in the Descemet s membrane and vague opacities in the stroma were still seen after three weeks He never returned for follow up In the last examination the left eye had visual acuity of 1 0

Case 2

A 36 year old drunken blacksmith who was arrested by the police Upon arrest he was violent and was incapacitated with an aerosol irritant projector from a distance of under one meter The patient sought ophthalmological help 48 hours after the attack He complained severe pain in the right eye

There was marked edema and erythema of the upper and lower lids of the right eye To examine the eye lid retractors were needed Upon arrival visual acuity in the right eye was 0 2

The cornea was almost totally denuded of epithelium Stromal edema was marked and many folds were seen in the Descemet s membrane In the anterior chamber a distinct flare and cells were present There was miosis and iris vessels were dilatated

The initial topical treatment consisted of atropin and chloramphenicol On the third hospital day corticosteroid was added to the local medication The patient was hospitalized 19 days Reepithelialization of the cornea was not complete until the 30th day with mild stromal edema pseudopterygium and a small scar in the nasal periphery of the cornea After three months visual acuity in the right eye was 1 25 The peripheral scar and small conjunctival overgrowth onto the cornea were still to be seen

Case 3

A 40 year old carpenter who was shot by a policeman with a tear gas solvent spray against the left eye at a near distance He admitted the hospital 20 hours thereafter

Upon arrival the right eye was intact The left eye was occluded by the largely swollen lids The conjunctiva was chemotic and the cornea was totally denuded of epithelium Stromal edema was marked many folds were seen in Descemet s membrane and the central part of the cornea was cloudy Visual acuity of the injured eye was one meter finger counting There was a moderate reaction in the anterior chamber

Atropin and chloramphenicol were started topically On the third hospital day local corticosteroid was added

On the 11th day he left the hospital with visual acuity of 0 2 in the left eye Regeneration of the corneal epithelium was complete but there were still stromal edema and Descemet s folds in the left eye By the third week with still opacity in corneal stroma and Descemet s folds he had visual acuity of 0 7 in the left eye The patient did not admit further controls

Case 4

A 27 year old metal worker who was shot with an aerosol irritant projector against the right eye from a distance of about 50 cm When seen 24 hours later the left eye

was intact but markedly swollen and erythematous lids occluded the right eye Visual acuity was hand movements in the injured eye The bulbar conjunctiva was chemotic Corneal epithelium was totally denuded Corneal stroma was swollen and its central and lower parts were strongly opacified The anterior chamber and other deeper parts could not be seen. Topical atropin and chloramphenicol + corticosteroid were started By the second hospital day epithelium the stromal haze and edema appeared to be clearing The degree of anterior chamber reaction was mild The patient was hospitalized 13 days Upon leaving the cornea was reepithelialized the punctate staining with fluorescein and small opacifications in the stroma were observed Local corticosteroid treatment was continued up to 4 months when visual acuity was 1 25 in the right eye In the cornea small opacifications were still present

Case 5

A 42 year old drunken metal worker who was arrested by the police Because of violence he was incapacitated with a near range shot of a solvent spray type of tear gas weapon He admitted the hospital 24 hours later There was marked swelling of the upper and lower lids of both eyes Lid retractors were needed to examine the eyes

Pus was abundant on the chemotic conjunctiva of both eyes Medication with topical atropin and chloramphenicol + corticosteroid was started The following day it was possible to perform a better ocular examination Slit lamp examination of both eyes showed a large epithelial abrasion in the lower halves of the cornea and conjunctiva moderate stromal edema and folds in Descemet s membrane The anterior chamber reaction was mild in both eyes

By the 8th day when the patient was discharged the left eye had totally recovered. In the right eye visual acuity was 0 6 because of cloudiness in stroma. Topical corticosteroid treatment was continued up to four weeks when also the right cornea had achieved its normal transparency

Case 6

A 40 year old drunken carpenter who was incapacitated by the police with a near shot of a tear gas pistol He sought ophthalmological help 12 hours after the attack

Examination revealed moderate swelling and redness of the skin in the upper part of the face Multiple grey black powder particles were imbedded in conjunctiva and superficial cornea of both eyes The stroma was mildly edematous and there were folds in Descemet s membrane The degree of anterior chamber reaction was mild in both eyes The conjunctivas were chemotic and the corneas were totally denuded of epithelium

Some of the superficial foreign bodies were removed mechanically and local medication with atropin chloramphenicol and corticosteroid as well as systemic penicillin were started

By two weeks with visual acuity of 0 9 the right cornea was reepithelialized but punctate stainability with fluorescein and minimal opacity in stroma was observed. More marked stainability and opacity was seen in the cornea of the left eye which had visual acuity of 0 3 At the last examination 5 months after the injury there was still some opacification in the left cornea The left eye had visual acuity of 0 8 The right eye was fully recovered

Discussion

The 6 patients described had typical lesions not greatly varying in severity at the first examination Lid and conjunctival edema and erytheam corneal changes and anterior chamber irritation were present The corneal epithelium in each affected eye was denuded and slowly regenerated in periods ranging from 8 days to some months Stromal edema also resolved slowly whereas anterior chamber reaction disappeared more quickly Five patients had corneal opacifications at the last follow up examination ranging from 3 weeks to 5 months

Treatment consisted of debridgement of foreign bodies when indicated and topical administration of cycloplegics antibiotics and corticosteroids All the patients recovered useful vision

Whereas several other studies have pointed out the ocular injuries caused by the explosive type of tear gas hand weapons this paper describes 5 patients out of 6 who were shot at close range with a nonexplosive type of tear gas weapon and had moderate ocular injuries Their lesions did not differ from those caused by a near shot with a tear gas pistol to one of our patients

Our findings well agree with those obtained in animal experiments MasLeod (1969) reported that a direct contact to the eye of liquid material obtained from an aerosol irritant projector caused severe and lasting damage to the eyes of rabbits and monkeys If the animals were anesthetized before the liquid was applied the reaction was even much more severe Corneal epithelium was lost evere stromal edema and corneal clouding persisting for 3 months and cornea' vascularization developed He as well as Macrae et al (1970) points out that aerosol irritant projector must no be discharged from less than 3 m otherwise chances of ocular damage considerably increase The great impor tance of self protective reflexes are emphasized by both

The possibilities for ocular damage in our cases were most favorable All the patients were drunken and it can be assumed that their reflexes for self protection were diminished Also tear gas weapons were wrongly used shot distance was much shorter than recommended and immediate first aid was not given All the patients came for treatment 12–48 h after the attack upon their own initiative

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TREATMENT OF TRAUMATIC HYPHAEMA

BY

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A series of 131 successive hospitalized cases of traumatic hyphaema after blunt injury of the eye were treated with complete bed rest for 6 days and double eye patching but without any topical medication Seven patients (5 3 %) had a secondary bleeding The visual acuities of 115 patients were tested and 105 regained their normal vision, i e 1 0 or over These results are no better than those reported in series treated with moderate bed rest for 3–4 days combined with topical administration of corticosteroids and mydriatics The results indicate that our present treatment may be unnecessarily severe

Key word anterior chamber – hyphaema traumatic – haemorrhage secondary – therapy

During the last 20 years the treatment of traumatic hyphaema has been varied considerably in the different eye clinics A variety of therapeutic procedures has been tried in order to prevent the occurrence of secondary haemorrhage recognized as one of the most serious complications In some places a relatively liberal treatment is practised The patients remain in bed for 3–4 days with patching of the injured eye only and are allowed to eat and wash unaided From the beginning the injured eye is treated topically with a mydriatic 1–3 times a day and a corticosteroid 3–5 times a day

In other places the treatment of choice has been more restrictive comprising complete bed rest with either single or double eye patching or pinhole glasses

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for 4–6 days or until the original hyphaema has been totally absorbed No mydriatics or miotics have been given during the first 4–5 days

We have made a retrospective analysis of a series of 131 consecutive patients with traumatic hyphaema treated by strict bed rest in hospital and double eye patching in order to establish whether this trying and expensive treatment can be justified

Material and Methods

A total of 209 cases with a diagnosis of contusion of the eye were treated in our ophthalmic department from 1969 to 1972 All were hospitalized Cases without traumatic hyphaema with only a microscopic hyphaema (66 cases) with only retinal oedema or retinal bleeding (9 cases) and those admitted later than 24 h after the incidence of trauma (3 cases) were excluded leaving 131 cases

The initial therapeutic regime for all patients was binocular patching and complete bed rest for 5–6 days or longer until the original hyphaema had disappeared No topical medications were administered routinely Local antibiotics were given in cases with severe corneal erosion Acetazolamide was used if an increased intraocular pressure (usually > 25 mmHg) developed Surgery was never resorted to for evacuation of blood or reduction of intraocular pressure

The following parameters were compiled from the records age sex size of initial hyphaema visual acuity at time of admission and after 6 weeks when tears of the ora serrata were also searched for duration of bed rest duration of treatment and all complications following original and secondary bleeding episodes

Results

Sex and age

The age and sex distribution is shown in Fig 1 More than half the patients (86) were younger than 20 years of age with as expected a preponderance of males

Original hyphaema

The cases were divided into three groups those with hyphaemas of 1–3 mm those with 4–6 mm and those with 6 mm or more (Fig 2) Only 15 patients

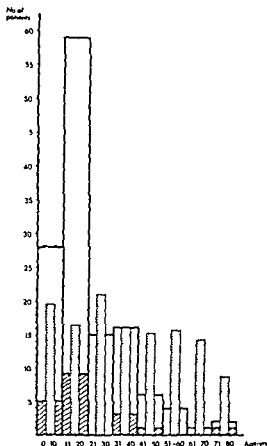


Fig 1
Age and sex distribution of the patients Open bars males Hatched bars females Dotted bars expected incidence of traumatic hyphaema if the diagnosis had been equally distributed throughout the population

had large hyphaemas i e ≥ 4 mm and the remainder (116 patients) had small hyphaemas

Visual acuity

In Fig 3 the visual acuity at time of admission is compared with the visual acuity 6 weeks later The visual acuities of 115 patients were tested and 105 regained their normal vision i e 1 0 or over one ended with visual impairment reduced to light perception one with 0 2 and the remaining eight with a visual acuity of between 0 4–0 9

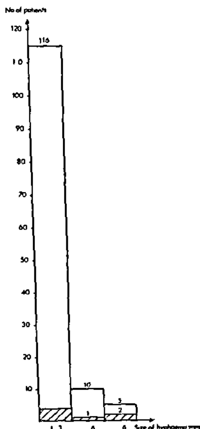


Fig 2

Number of patients (open bars) with 1–3 mm 4–6 mm and > 6 mm primary hyphaema
Number of patients (hatched bars) with secondary hyphaema in the respective groups

Tears of the ora serrata

After 6 weeks 72 patients were examined for tears of the ora serrata with an indentation contact glass In only one case was there a small tear of the ora serrata and this did not require treatment

Bed rest

The average duration of bed rest was 5 8 days after patients with bed rest ≥ 10 days (8 patients) had been excluded The average duration of the stay in hospital (of those who were hospitalized for less than 10 days) was 6 2 days

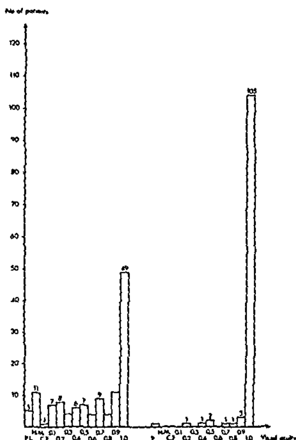


Fig 3

Visual acuity on admission (left) and after 6 weeks (right) Visual acuity in decimal fractions when tested on Monoyer letter chart P I = ability to have perception of light H M = ability to appreciate movements of the hand C F = ability to count fingers

Thirteen patients stayed in hospital for 10 days or more The reasons for these prolonged hospitalizations are listed in Table I The total average stay was 7 1 days

Secondary haemorrhage

Seven cases had a second haemorrhage (5 3 %) (4 1 % of all cases with microscopic and macroscopic anterior chamber bleeding 3 8 % of all cases with the diagnosis of contusio bulbi) The secondary haemorrhage occurred on the 3rd to 7th day (Table II) No common factor could be found to explain why

Table I
Reasons for prolonged hospitalization.

	No of patients
Secondary bleeding	5
Suspected perforation	1
Corneal erosion	1
Iritis	1
Delayed blood absorption	1
Increased intraocular pressure	2
Choroidal rupture and vitreous haemorrhage	1
Enucleation	1

3 out of 7 secondary haemorrhages occurred so unexpectedly late as on the 7th day

The primary chamber bleeding had in 4 cases been ≤ 3 mm 6 mm in one case and in two cases it filled the anterior chamber (Fig 2) In two cases the secondary haemorrhage recurred after 2 days One of these cases ended with an increased intraocular pressure (IOP) and an impaired vision reduced to light perception only One case ended with an increased IOP and a traumatic cataract with a visual acuity of 0 2 In the other cases the secondary haemorrhage was spontaneously absorbed with no permanent visual impairment *Other associated lesions* are listed in Table III These complications reduced the vision to 0 2 in a patient with iridodialysis and cataract to 0 5 in a patient with dislocated lens cataract and increased IOP to 0 4 in a patient with choroidal rupture and to 0 8 in a patient with choroidal rupture and ablatio retinae No other complications resulted in impaired vision The enu

Table II
Appearance of secondary hyphaemas

Day	3rd	4th	5th	6th	7th
No of patients	1	1	1	1	3

Table III
Associated lesions

	No of patients
Corneal erosion	2
Iritis at discharge	26
Cataract	2
Dislocated lens	1
Increased intraocular pressure	3
Iridodialysis	3
Vitreous or/and retinal bleeding	6
Detachment of retina	1
Retinal hole	2
Tears of ora serrata	1

cleation was undertaken because of a large hyphaema that did not reabsorb a large iridodialysis and visual acuity of light perception only

The 78 patients that were not accepted for more detailed analysis in this study were also treated with complete bed rest and double eye patching for 5–6 days In this group of patients there was one secondary haemorrhage which occurred on the 3rd day and which was reabsorbed without any complications One case ended with a visual acuity of 0 9 as a result of a choroidal rupture and in all other cases full vision (*i e* 1 0 or over) was regained

Discussion

The data on age and sex distribution in our series correspond to those in previous studies with a gross preponderance of young persons especially boys This is in agreement with most other studies but apart from this the variability between different studies is noteworthy In most of our patients (116/131 = 89 %) the original hyphaema was small (≤4 mm) The percentage of secondary hyphaema was 5 3 % In some of the series where the hyphaemas have been graded the percentage of large hyphaemas is higher Oksala (1967) reported 35 % and secondary bleedings occurred in 5 7 % of this series Edwards &

Layden (1973) found 51 % large hyphaemas and 35 % secondary hyphaemas Some other materials show a preponderance of small hyphaemas In the prospective study by E Eagling (1974) the hyphaemas were microscopic in 37 % and the percentage of secondary hyphaemas was 18 % Gregersen (1962) reported mainly small hyphaemas in 80 % and 5–6 % secondary bleedings Edward & Layden (1973) indicated a direct correlation between the size of the primary hyphaema and the occurrence of secondary bleeding and the complication rate respectively They also suggested that the favourable course reported in many papers seems to be due to the preponderance of small primary hyphaemas in these materials The figures cited above tend to support this although there are exceptions

In the papers from eye clinics in USA there is generally a much higher incidence of secondary bleedings (12–35 %) than in ours although the mode of treatment seems to be the same (see Shea 1957 Kushner 1959 Henry 1960 Cole & Byron 1964 Sheie Sowa & Grayson 1972 Edward & Layden 1973) In their materials other complications are also more numerous indicating that their materials are presumably composed of patients with more severe contusions than in our series Gregersen (1962) also noted that it seems likely that differences in referral and admission standards are responsible for the great discrepancies in the frequency of complications that exist in different studies We have therefore tried to compare our results with those of clinics where the referral and admission practice can be expected to be similar to ours

Öhrstrom (1972) reported a retrospective study of 167 patients with hyphaema in the neighbouring clinic of Malmö These patients were referred and admitted according to the same principles as those in our material They were treated with bed rest for 3–4 days and bandaging of the injured eye only Mydriatics and corticosteroids were given topically The amount of primary hyphaema was not graded but the rate of other complications seems to have been about the same as in our series The incidence of secondary bleedings was only 2 4 % and of secondary iritis 1 8 %

In Stockholm Zetterstrom (1969) carried out a prospective study of 117 patients with contusion No comment about the primary bleeding is made but probably some cases without hyphaema are included in this material All patients were hospitalized but half of them (Group I) were treated by strict measures similar to our type of treatment and half (Group II) were allowed to move about freely and were given mydriatics and corticosteroids locally Four cases of secondary hyphaema (6 7 %) occurred in group I and none in group II

The number of other complications in the two groups and in our series is about the same The patients in group II did not show any symptoms of sec

ondary iritis on their discharge but 18 6 % of the patients did so in group I In our series 19 2 % of the patients had a secondary iritis on their discharge The patients in group I were in hospital on average for 6 3 days (in our series 6 2 days) the corresponding figure in group II being 4 8 days

The great discrepancies between the incidence of secondary bleedings reported in different studies emphasize that far reaching and detailed conclusions cannot be drawn without carefully controlled studies However our restrictive type of treatment is very expensive and trying both to the patient and the wardstaff We feel that its disadvantages are so great that the advantages needed to counterbalance them should at least be large enough to become apparent in this retrospective study of 131 cases and without the support of an impeccable control group The lower 95 per cent confidence limit for secondary bleedings is in our material 2 0 % and it is thus unlikely (at the 5 per cent probability level) that our true bleeding incidence is lower than 2 % This is no better than in the results presented by Zetterstrom (1969) and Ohrstrom (1972) who employ a much less severe and less expensive treatment but with similar patient selection standards We find ourselves in doubt about the value of our present treatment policy

On the other hand it is worth noticing that if our incidence of bleeding is compared by χ analysis with that of Zetterstrom s group II or Ohrstrom s patients or both combined (assuming comparable patient selection procedures) the difference is not statistically significant even in a one tailed test indicating that their type of treatment does not necessarily result in fewer bleedings than ours

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Holmes William John (ed) Documenta Ophthalmologica Proceedings Series Volume 5 Conference for the Prevention of Impaired Vision and Blindness Paris 1974 ISBN 90 6193 145 2 Price Dutch Guilders 65 –

Blindness in the world to day

The human being is a social animal but it accepts responsibility only to the individual tribe This is clearly borne out by the Report of the Conference for the Prevention of Impaired Vision and Blindness which was held in Paris in 1974

While in the Western world blind people are rarely encountered (incidence in the U K 200 per 100 000) in other countries such as the Cameroons up to 14 % of males over 20 years of age become blind from onchocerciasis (Anderson and Fuglsang) The incidence varies with the distance from the infected rivers but there is probably also an individual variation in the tendency to severe ocular involvement This has given rise to a search for vaccines against the larvae (Mojon) So far however treatment of the infected persons is the only solution Anderson and Fuglsang and Beiram report convincing results of treatment with diethylcarbamazine and suramin Unfortunately these drugs give vertigo lymphadenopathy so severe as to make walking almost impossible itching stomatitis dermatitis and iritis Thus it is no wonder that chemo therapy is unpopular Some of the reactions are caused by the death of large numbers of microfilaria in the skin lymph nodes and eyes but large scale pharmacological research to introduce drugs with fewer adverse reactions are highly warranted They may not give immediate pay off to the pharmaceutical firms but the long term social profit is certain

Hypovitaminosis A which is of course also preventable is the commonest cause of paediatric blindness in India where according to Roy and Ahmed 40 % of 4 39 million blind children had keratomalacia and 30 % of children from Bangladesh located in a refugee camp showed signs of hypovitaminosis A This was particularly prevalent in children with intestinal worms

Chandra and Kumar reported on visual screening of 74 620 persons from the rural districts of North Central India and found 2 074 blind people per 100 000 Conjunctival infections including trachoma were the most important cause but of the causes of blindness revealed only 9 09 % were neither curable nor preventable

Indonesia is on her way to better standards of living because here there is money at least to make a census In a country where 90 % of the houses do not have a toilet and 70 % have no floor it is surprising that the prevalence of blindness is only 800 per 100 000 even if blindness supposedly for economic reasons is defined as vision of 1/60 in the better eye (Madang)

Kuang Hui Lim of Singapore reports that the prevalence of blindness (< 3 60) is 670 per 100 000 but that trachoma and xerophthalmia are now virtually eradicated reflecting as the author said improvement in the health of the citizens and the gain in the economy of the country

The population explosion has increased the shortage of ophthalmic man power in the developing world According to Kirmani some people living on the outskirts of Karachi had to wait four or five years before they could have their eyes operated on. During the last 20 years that city has increased from 300 000 to 4 millions

And so this report continues demonstrating unnecessary blindness among the poor nations Sir John Wilson in his challenging address on Mobilization of Public Opinion in the Public Health Ophthalmology estimates that by the end of this century 30 millions will be blind

In the Western world the prevention of hereditary disorders diabetic retinopathy glaucoma and monocular amblyopia constitute the main problems and these were discussed at the conference The problems of prevention of blindness in this part of the world is obviously important to the people living there but they represent only a small office in the building of contemporary ophthalmology and we must find service men for the other rooms Michaelson s Jerusalem Institute for the Prevention of Blindness and Ocular Disease is one way of attacking the problem Nizetic of the WHO describes the possibilities of educating the public training ophthalmic assistants and improving the instruction of general practitioners so that the ophthalmologists can be utilized as effectively as possible

Did any one suggest that three months work in a developing country should be obligatory for doctors who want to practise ophthalmology?

Mette Warburg

Moses Robert A (ed) Adler s Physiology of the Eye Clinical application Sixth edition The C V Mosby Company Saint Louis 1975 70[illegible] pages 6[illegible]4 illustrations Price US dollars 28 90

Ever[illegible] years since 19[illegible]0 a new edition of Adler s physiology has appeared In the sixth edition the number of co authors has increased to 17 as compared to 5 in the 19[illegible] edition The natural consequence of this policy has been a profound revision of most of the chapters In spite of this it has been possible to keep the number of pages unchanged and the number of chapters remains 23 Each chapter is concluded with several references (on an average [illegible]) and often a list of general references is provided in addition As indicated by the title the aim of the book is to link the eye clinic with eye physiology in this it has succeeded to a high degree In each chapter the authors list and elaborate a large number of relevant clinical data and methods by placing them into a functional relationship with basic physiological and anatomical facts

This two sidedness of the text together with a relatively concentrated presentation means that the book does not penetrate deeply into purely physiological problems Probably it appeals chiefly to clinicists with a general interest in the ophthalmic function or as a first introduction into a special topic for those interested in ophthalmic research Due to its clinical application Adler s Physiology is a valuable textbook in the education of future eye specialists The aim of the book is well fulfilled it is highly recommended

Ole I Nissen

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2nd International Visual Field Symposium

will take place in Tubingen W Germany (19–22 September 1976) Open to all members of the International Perimetric Society and their guests For all information contact Dr E L Greve Eye Clinic University of Amsterdam Wilhelmina Gasthuis Eerste Helmerstraat 104 Amsterdam–1013 The Netherlands

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will take place May 19 to 23 1976 in Southhampton Princess Hotel Bermuda The program will include discussions and panels on strabismus genetics metabolic diseases surgical advances and other aspects of pediatric ophthalmology For information Edward L Raab M D Department of Ophthalmology Mount Sinai School of Medicine Fifth Avenue and 100th Street New York New York 10029